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CONTENTS

Abstracts of papers accepted for presentation at the forty-sixth annual meeting of the American Phytopathological Society, Estes Park, Colorado, August 25, 26, 27, 1954 481

Listing of books 512

The mechanism of wilting in tobacco plants affected by black shank
HARRY R. POWERS, JR. 513

Antagonism in soil between Macrophomina phaseoli and selected soil inhabiting organisms
DON C. NORTON 522

A rapid spectrophotometric assay for some plant viruses
HUGH C. KIRKPATRICK AND R. C. LINDNER 525

Studies concerning chemotherapy of two plant viruses
HUGH C. KIRKPATRICK AND R. C. LINDNER 529

Studies on stone-fruit viruses in cucurbit hosts. IV. Some effects of hydrogen ion concentration and dilution on infectivity R. S. WILLISON AND M. WEINTRAUB 533

Studies on stone-fruit viruses in cucurbit hosts. V. Thermal inactivation of some isolates
M. WEINTRAUB AND R. S. WILLISON 538

Meadow nematodes associated with failure of spring-sown alfalfa RICHARD A. CHAPMAN 542

The inhibitive effect of water in infection by tobacco mosaic virus
WILLIAM B. ALLINGTON AND EDWARD F. LAIRD, JR. 546

The effect of a phytophagous species of Tetrastichus, new to the United States, on sweet clover infected with wound-tumor virus SHIRLEY S. TEITELBAUM AND L. M. BLACK 548

Symptoms on tomato fruits incited by three Phytophthora species P. D. CRITOPOULOS 551

A Phytopathological Note:
Observations on the age of lodgepole pine tissues susceptible to infection by Arceuthobium americanum FRANK G. HAWKSWORTH 552

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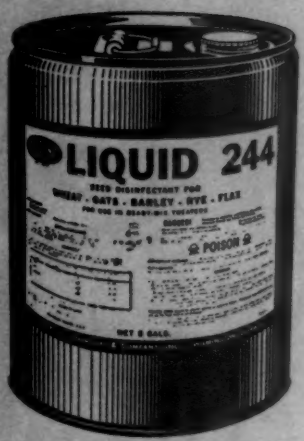

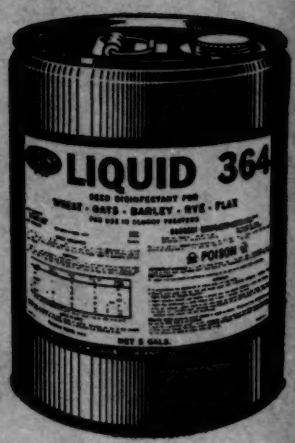

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Spread of Lambert mottle virosis of sweet cherry in Montana. AFANASIEV, M. M., AND H. E. MORRIS. A virus disease which closely resembles Lambert mottle is the most important virus disease of cherry in Montana. Positive transmission was obtained with this disease by bud-inoculation. At the present time the disease is found only on Lambert and Peerless varieties of sweet cherry. In individual orchards, the virosis spreads radially from the original sources of infection. Infected trees in the orchards were found in groups, which indicates that transfer of virus takes place from one tree to the next rather than over great distances. Occasionally an infected tree is found at a considerable distance from any apparent source, but the spread from this kind of tree is always to an adjacent tree. This type of spread indicates that the vector of this virosis has a limited range of movement. This disease causes definite economic losses in the orchards since very often infected trees die in 3 years.

Behavior of isolates of race 59 of Puccinia graminis tritici on certain varieties of wheat and barley. ALI, SYED B. Race 59 of *Puccinia graminis tritici* comprises at least 4 biotypes, designated 59, 59A, 59B, and 59C, differentiated only on the resistant Marquis, Reliance, and Kota wheats. All biotypes produce infection type 2 on Marquis. On Reliance, 59 and 59C produce type 0, and 59A and 59B produce type 2. On Kota, 59 and 59A produce type 0; whereas 59B and 59C produce type 2. From 65° to 90°F., infection types 0-0 remain stable; at 90+ °F., type 2 changes into an atypical 3 or 4 with profuse sporulation but with early chlorosis and a necrotic center in each uredium, evident on the lower leaf surface. Race 59C closely resembles race 1 at 90° F. and differentiation is difficult. On additional hosts, biotypes can be differentiated at 90° F.; Kenya 117A wheat is susceptible to 59B and resistant to other biotypes. Mars, Moore, and Plains barley are resistant to 59C but susceptible to other biotypes. Further specialization within the race occurs, since Kentana 51A wheat is susceptible to 1 isolate each of 59B and 59C but resistant to other isolates of both. Similarly, Peatland, Feebar, and Minn 615 barley are susceptible to 1 isolate of 59C but resistant to another.

Inheritance of resistance to the variant strain of the common bean mosaic virus. ANDERSEN, AXEL L. AND E. E. DOWN. Studies on inheritance of resistance to the variant strain of common bean mosaic virus indicate that resistance in U. S. No. 5 Refugee and Trag 279-1 (a Mexican introduction) is governed by a single dominant gene in these 2 varieties. This observation is based upon the reaction of crosses between these 2 varieties as well as the F_1, BC_1, and F_2 generations resulting from crosses of these 2 resistant varieties and 6 susceptible varieties including Michelite, Cranberry, and Dark Red Kidney. On the other hand, crosses between Great Northern U.I. No. 31, a resistant variety, and the 6 susceptible varieties gave 2 different types of reaction, depending upon the resistance of the susceptible parent to common bean mosaic virus. Thus virus 1-resistant bean plants crossed with Great Northern U.I. 31 produced F_1 plants resistant to mosaic and F_2 plants that segregated 3 resistant: 1 diseased, indicating that resistance is governed by a dominant gene. Bean virus 1-susceptible plants crossed with Great Northern U.I. 31 produced F_1 and BC_1 plants that were susceptible and the F_2 generation segregated 1 resistant: 3 susceptible, which indicates that resistance is governed by a recessive gene.

Antibiotics as protectants against fireblight. ARK, PETER A., AND C. EMLEN SCOTT. Bartlett pear trees sprayed with 100 p.p.m. crude streptomycin at 25 per cent bloom, 35 per cent full bloom, calyx, and 3 additional times, gave 0.602 cuts for blight per tree, as against 0.688, 2.09, 0.645, and 0.924 cuts per tree with copper-lime dust, untreated, 50 and 25 p.p.m. streptomycin respectively. In another trial, spraying pears with streptomycin at 100, 25, and 10 p.p.m. gave 0.02, 0.49, and 6.9 cuts per tree as compared to 6.94 cuts in the check. Where streptomycin (100 p.p.m.) was sprayed on half of each of 8 Jonathan apple trees at full bloom, petal fall and calyx showed there were 15 blight infections compared to 91 in the unsprayed halves. Streptomycin dust (240 p.p.m.) showed greater variation in protection than copper-lime dust, which is the standard blight control in most California pear districts. Trunk injections of dry streptomycin up to 3.6 gm. (base) per tree failed to check holdover cankers but caused no apparent injury. Neomycin sprays at 50, 25, and 10 p.p.m. showed 2.77, 4.45, and 4.15 cuts per tree as against 10.21 cuts in the check. Aureomycin at 25 p.p.m. applied 4 times showed 8.48 cuts as compared to 4.22 cuts per tree in the corresponding checks.

A study of corynebacterium sepedonicum as related to host resistance and spread by water. ARNOLD, GORDON W. Ring rot of potatoes caused by *Corynebacterium sepedonicum* remains a serious threat to the potato industry. The bacterium was grown on media containing extracts from varieties susceptible and resistant to ring rot. No consistent differences were noted in ability of the various media to support growth of the organism. This suggests that the factor for ring rot resistance of potato is not necessarily connected with or a constituent of the juice. Water suspensions of the organism were applied to the soil of potted greenhouse potato plants. Plant symptoms and Gram stain technique were used to determine the degree of infection. Potted plants receiving 6 weekly applications of inoculum plus soil stabs had the greatest disease incidence. Regardless of the system of inoculation used, all treatments caused plant infection. These data suggest that water can be a factor in spread of ring rot and that the chance of infection is greatly increased if underground portions of the potato plants are damaged before coming into contact with water in which bacteria are present.

Control of several small grain diseases by the water soak method. ARNY, D. C., AND CURT LEBEN. In greenhouse and field tests the Tyner soaking method was used for seed treatment. Seed was soaked in distilled water for 56 hours at 22–24° C. In greenhouse tests the soaking method was nearly as effective as Ceresan M (ethyl mercury p-toluene sulfonanilide 7.7 per cent) for the control of seedling blights of barley and oats, caused by *Helminthosporium sativum* and *H. victoriae*, respectively. There was excellent control of the loose smut of barley where infection was moderate; the presence of the antibacterial antibiotics, aureomycin, streptomycin sulfate, or terramycin in the soak water did not lessen control. In most of the barley samples the soaking treatment reduced germination. When barley and oat seeds were placed on an agar medium, those that had been soaked gave rise to fewer *Alternaria* and *Helminthosporium* colonies than unsoaked seeds.

Effect of air temperature on multiplication of tobacco mosaic virus in tobacco. BANCROFT, JOHN B., AND GLENN S. POUND. The concentration of TMV was determined in leaf extracts of susceptible Connecticut Havana No. 38 tobacco plants and of plants of a tobacco breeding line containing the Ambalema genes for resistance, grown at 16°, 20°, 24°, and 28° C. At 4 days after inoculation, a sharp gradient of virus concentration existed in inoculated leaves of the susceptible variety, the concentration increasing with increase in temperature. Consecutive weekly assays from systemically-infected tip leaves of the susceptible variety revealed a cyclic phenomenon. Maximum concentrations were obtained consecutively at 28°, 24°, 20°, 16°, 28°, 28°,

24°. Virus titres were directly correlated with symptom severity. With extracts taken from all leaf tissue of treatment samples, a somewhat similar cyclic phenomenon was evident. This phenomenon was not evident in assays from resistant Ambalema plants. With these plants, resistance was expressed at 16° and 20° and virus multiplication in them was low. Resistance broke down at approximately 22°. Slight symptoms at 24° corresponded with increased virus multiplication. At 28° severe symptoms were expressed and virus concentration was moderately high.

Biological control of Fusarium wilt of sweet potato. Bega, Robert. Greenhouse and field trials were made to test the effect of the sweet potato sprout rot Fusarium (*Fusarium solani* f. *batatas* McClure) against the wilt organism (*Fusarium oxysporum* f. *batatas* (Wr.) Snyd. & Hans.). Both organisms are wound parasites unable to infect uninjured tissues. By use of a double inoculation method (dipping the freshly cut basal end of sprouts, first in a spore suspension of the sprout rot organism, and, after various time intervals, in a spore suspension of the wilt organism) greenhouse tests have shown that the limited basal rot caused by the sprout rot Fusarium will protect the plant against subsequent invasion by the wilt Fusarium. Field tests, made by dipping freshly cut sprouts in a spore suspension of the sprout rot organism and then planting in infected soil, have shown that the same protection noted under greenhouse conditions is also found to be effective in the field.

Serological reactions of a plant virus transmitted by leafhoppers. Black, L. M., and M. K. Brakke. Wound-tumor virus was highly purified by zonal centrifugation and zonal electrophoresis in sucrose solution density-gradient columns. The virus was pelleted by high speed centrifugation and resuspended in small volumes of 0.85 per cent NaCl buffered at pH 7.3 with 0.05 M phosphate. The suspension was emulsified with an equal volume of a 9:1 mixture of Mayol F mineral oil and Arlacel A emulsifier. Rabbits were given 1 to 4 injections of these preparations in the large muscles of the hind legs. Virus isolated from plants, *Melilotus alba*, or from insects, *Agallia constricta*, produced antisera reacting with purified virus from the opposite source at serum dilutions up to 1:1600, indicating common antigenic reactions for virus obtained from the 2 sources. Precipitin, complement-fixation, and specific neutralization reactions were demonstrated, the latter by injection of serum-virus mixtures into leafhopper vectors. These techniques provide a rapid assay and means of determining relationships among some leafhopper-borne viruses. In plants and insects invaded by the virus, but not in virus-free hosts, a second substance much smaller than the virus occurred which reacted with the antiserum. Purified virus broke down at a rate dependent on temperature and pH to give a similar, perhaps identical, small antigen.

Synergistic effect of growth-regulating chemicals and tumefacient virus on plant cells. Black, L. M., and C. L. Lee. A mixture of 2 per cent alpha-naphthalene acetic acid in lanolin was applied with and without wounding to various nodes of *Melilotus officinalis*, clone C10, either healthy or systemically invaded by wound-tumor virus. In healthy plants, in addition to the other usual effects of this substance, callus tissue was visible within 1 week and adventitious roots within 2 weeks. Callus and roots enlarged slowly and the latter reached their maximum development of 1 mm. in 4 weeks. Adventitious roots continued to arise over a period of at least 8 weeks. Plants invaded by the virus reacted similarly during the first week but at the end of 2 weeks the overgrowth at the treated node, whether wounded or not, was much larger than in healthy plants and showed early stages of tumor formation. In addition many small tumors appeared in the adjacent internodes. Development of these tumors continued for many weeks and was more rapid than in plants with virus but no growth-regulating chemical. These tumors also differed from tumors on the latter in sometimes bearing small adventitious roots over portions of their surface. Indoleacetic acid produced similar but less marked effects.

Genetic, nutritional, and pathogenic studies with induced vitamin deficient mutants of Venturia inaequalis. Boone, D. M., H. A. Lamey, D. M. Kline, and G. W. Keitt. Induced mutants of *Venturia inaequalis* deficient, respectively, for biotin, nicotinic acid, pantothenic acid, inositol, choline, or riboflavin were studied. Each deficiency was inherited as if single-gene controlled and some of the mutant genes could be located with reference to their respective centromeres by the percentage of second-division segregation. Tests for synergism with 5 riboflavin mutants indicated that they represented mutations at 3 or more different loci; similar tests with 3 pantothenic acid, 3 inositol, and 5 choline mutants gave no evidence that more than 1 locus was concerned for each of these groups. Results from dry weight measurements of the growth-response of representative mutants to different concentrations of the required vitamins indicated that the deficiencies were complete and showed the minimum concentrations of the vitamins that would support growth. The mutants deficient for biotin, nicotinic acid, pantothenic acid, or inositol incited the typical lesion reaction of the *normal* line from which all the mutants here reported were derived, whereas the choline and riboflavin mutants merely incited pitting or flecking. Applications of a choline solution on leaves inoculated with a choline mutant enabled this mutant to develop and to incite the typical lesion reaction.

Penicillium sp. parasitic on Rhizoctonia solani. Boosalis, M. G. Field soil and roots of several crops submerged 1 minute in water (75° C.) yielded a *Penicillium* sp. parasitic on 25 isolates of *Rhizoctonia solani*. *R. solani* grown on sand-corn meal medium or on potato-dextrose agar (PDA) containing 20 gm. dextrose/l., was severely parasitized by the *Pencillium* under study. Yellow perithecia of the hyperparasite covered the surface of most *R. solani* cultures in 21 days. Although hyphae of *Penicillium* were not discerned inside all host mycelia, subsequent transfers from parasitized *Rhizoctonia* cultures resulted only in growth of the former. Relatively little damping-off and few necrotic lesions developed on peas planted in sterilized and non-sterilized field soil heavily infested with *Penicillium*-parasitized *R. solani*. Non-parasitized *R. solani*, however, caused 50 per cent of the peas to damp-off and incited deep lesions on 80 per cent of the seedlings. The hyperparasite was not pathogenic to peas. Parasitic activity of the *Penicillium* isolates was negligible on *R. solani* grown on PDA with 10 gm. dextrose/l. The parasitic fungus penetrates *R. solani* by infection hypha from coiling mycelium. *Fusarium* spp., *Sclerotinia sclerotiorum*, *Helminthosporium sativum*, *H. avenae*, *Pythium debaryanum*, and *Cercospora beticola* were not parasitized by *Penicillium* sp. Thermal death point of *Penicillium* sp. is 59.5° C.

Effect of temperature on the reaction of wheat seedlings to Puccinia graminis tritici. Bromfield, K. R. Seedling reactions of selected wheat varieties exhibiting a temperature induced breakdown in rust resistance were determined at 70, 77, and 85°F. Twenty varieties infected with race 17, 12 with race 38, and 15 with race 56 were resistant at 70°F. and susceptible at 77 and 85°F. Selected varieties infected with races 17, 38, and 56 were kept for different lengths of time up to 15 days after inoculation at 70°F. and then transferred to 77 and 85°F. The same varieties were also placed initially at 77 and 85°F. and then transferred periodically to 70°F. Resistance apparent at 70°F. was not retained in plants transferred to 77 and 85°F. Sporulation typical of that produced by mycelium growing in susceptible tissue subsequently occurred on the periphery of areas which were necrotic flecks at time of transfer. Pustule development was arrested on plants transferred from 77 and 85°F. to 70°F. A characteristic ring of necrotic tissue developed around these pustules. Selected varieties infected with races 17 and 56 were exposed daily to periods of 70 and 77°F. in various combinations to determine the minimum high temperature period required for breakdown of resistance.

Chemical modification of rust reaction of seedling wheat plants infected with Puccinia graminis tritici. BROMFIELD, K. R., AND C. E. PEET. Maleic hydrazide treated wheat seedlings were consistently more susceptible in stem rust reaction than similar untreated plants. In no case, except Khapli, was a resistant type reaction changed to susceptible. Immune reactions were not altered. Reaction types of the standard differential varieties and of Lee infected with races 17, 38, and 56 were raised by a plus or double plus. Plants were treated by spraying to the point of incipient run-off with 0.075-0.160 per cent maleic hydrazide solution 1-3 days following inoculation. Comparisons were made of the effect of maleic hydrazide and DDT (shown by T. Johnson to alter the resistance of Khapli to some races) on Khapli infected with races 11, 15B, 49, and 139. Occasional pustules on plants treated with an 0.08 per cent solution of maleic hydrazide exhibited secondary sporulation. Pustules on plants treated with 0.16 per cent DDT were infection types 3— to 3+. Reactions of untreated Khapli were infection type 1.

Breakdown of rust resistance in detached leaves of normally resistant oat varieties. BROWNING, J. ARTIE. Seedling leaves of the crown and stem rust differential oat varieties were inoculated with uredio spores of *Puccinia coronata avenae* and *P. graminis avenae*, detached and supplied with 2 per cent glucose or sucrose solutions in light and in darkness at about 70°F. Leaves from varieties normally susceptible to a given race of 1 of the rust organisms remained susceptible following the treatment; leaves from varieties normally resistant to a given race frequently became susceptible. This breakdown of rust resistance was sometimes more rapid and complete in darkness than in light. When the stem rust differential varieties were inoculated with *P. graminis avenae*, detached leaves of Richland and Minrus became susceptible to race 2, and Richland and Canuck became susceptible to race 7. When the crown rust differential varieties were inoculated with race 202 of *P. coronata avenae*, detached leaves from normally resistant Victoria, Landhafer, Santa Fe, Ukraine, Trispernia, Bondvic, and Saia gave susceptible reactions as did the normally susceptible varieties Anthony, Appler, and Bond.

Stem rust resistant oat strains derived from irradiated seeds. BROWNING, J. ARTIE, AND K. J. FREY. Four hundred seeds of stem rust-susceptible Huron oats were treated with 25,000 r units of X-ray. Forty-eight lines with desirable agronomic characteristics were propagated from 25 of these seeds and for the most part showed field resistance under stem rust epiphytotic conditions in 1953. In the fifth generation following irradiation these lines were tested in the seedling stage at about 70°F. with races 6, 7, 7A, and 8 of *Puccinia graminis avenae*. All lines were susceptible to race 6; 3 were also susceptible to races 7, 7A, and 8; 3 were susceptible to races 7 and 7A but were resistant to race 8; 3 were susceptible to races 7 and 7A and were segregating for resistance to race 8; 37 lines were resistant to races 7 and 7A but were susceptible to race 8; and 1 line was segregating for resistance to races 7, 7A, and 8.

A leaf and twig blight of English holly caused by a species of Phytophthora. BUDDENHAGEN, IVAN W., AND ROY A. YOUNG. A new disease of English holly (*Ilex aquifolium* L.) caused by a species of *Phytophthora* occurs in holly orchards and nurseries in Oregon and Washington. The first symptoms are the appearance of black marginal leaf spots and subsequent defoliation resulting from ethylene produced by diseased leaf tissue. Leaf spots appear first on the lower parts of the trees in late fall, and infection continues upward during cool winter weather. Twig dieback, development of cankers, and infection of berry clusters occur during winter and early spring. Sporangia borne in leaf spots provide inoculum for subsequent leaf and twig infections. At 5–15°C. sporangia germinate primarily by zoospores; at higher temperatures germination is primarily by germ tubes. The disease may become epiphytotic during cool, rainy periods but is inactive during the dry summer. Oospores formed in the leaves and stems may provide the over-summering stage. Stem cankers and defoliation previously attributed to *Boydia insculpta* (Oud.) Grove and *Phomopsis crustosa* Bomm. & Rouss. are now considered to be caused by the *Phytophthora*. The *Phytophthora* pathogen belongs in the Syringae group of Leonian and most closely resembles *P. hibernalis* Carne, but apparently is distinct from any described species of *Phytophthora*.

Pathogenicity of Fusarium solani f. phaseoli in different soils. BURKE, DOUGLAS W. Pure-culture or infested-soil inocula of *Fusarium solani* f. *phaseoli*, in concentrations sufficient to cause severe bean rot in a virgin soil in the greenhouse, produced much less damage in soil from a root-rot-free bean field. This pathogen-suppressing property of the latter soil could be removed by heat or chemical sterilization, indicating that it was microbiological in nature. However, a microflora analysis revealed no specific differences correlated with this property. Attempts to establish this disease-suppressing factor in other soils by transfer of soil, microorganisms, and plants, were unsuccessful. Further direct studies of buried spores on water agar indicated the mechanism by which the inoculum potential of the pathogen may be depressed and also the state in which this *Fusarium* may survive in the soil. The root-rot-free soil favored germ-tube growth and mycelial development with comparatively late formation of small, terminal chlamydospores. Conversely, in a naturaly-infected soil germ-tube growth was suppressed and numerous large chlamydospores were formed within the macrospores as well as terminally on the germ tubes. Lysis of mycelium and germ tubes then occurred within 6 days in both soils so that the organism survived only as chlamydospores, which were larger and more numerous in the naturally-infested soil.

Transmission of a growth-retarding factor from old-line to young-line Eureka lemon trees. CALAVAN, E. C., J. M. WALLACE, AND L. G. WEATHERS. Inoculation of young-line (nucellar) Eureka lemon trees, in 1948 and 1949, with buds and spur buds from declining or collapsing old-line Eureka lemon trees caused significant retardation of growth, in comparison with non-inoculated controls, in 3 to 5 years. Growth retardation occurred in young-line Eureka on 3 rootstocks: grapefruit, sour orange, and sweet orange. Differences in trunk circumference growth between inoculated trees and paired controls from January, 1950, to January, 1954, averaged approximately 1 in. for young-line Eureka trees on grapefruit or sour orange and ½ in. for those on sweet orange rootstocks. Differences in growth during 1953 between inoculated and control trees of these 3 combinations are statistically significant at odds of 99 to 1. Trunk growth of inoculated old-line Eureka trees during the past 4 years was equal to that of their controls. Ledig Lisbon lemon trees were not significantly retarded in growth by inoculation. Quick decline virus did not appear to be present in any of the numerous old-line Eureka sources used for inoculum. It is concluded that an infectious agent, probably a virus, is at least partly responsible for the poor vigor of old-line Eureka lemon trees.

Knox, a new leaf rust resistant and stem rust escaping soft winter wheat. CALDWELL, RALPH M., LEROY E. COMPTON, JOHN F. SCHAFER, AND FRED L. PATTERSON. A soft, red winter wheat variety possessing resistance to leaf rust, *Puccinia rubigo-vera tritici*, was released to seedsmen in September, 1953, under the name "Knox." At Lafayette, Indiana, under artificially induced field epiphytotics of several races of leaf rust, it has incurred less than 1 per cent average infection and has expressed a highly resistant reaction type as compared with over 90 per cent infection of the susceptible Trumbull variety during the period 1948 to 1953. The resistance to leaf rust was derived from the spring wheat Chinese C.I. 6223 through a complex series of crosses initiated in 1927. This resistance is of the "mature plant" type, the seedlings being fully susceptible to all races thus far tested. Being 7 to 14 days earlier than

standard varieties in the central soft wheat area, Knox also possesses a significant capacity to mature before the development of destructive epiphytotics of stem rust, *Puccinia graminis tritici*. This advantage was demonstrated in a severe natural outbreak of stem rust in southern Indiana in 1948. These characters are associated with yield and milling and baking qualities superior to those of commercial varieties currently being grown in the central soft wheat region.

A bark splitting virus disease of Montmorency sour cherry. CAMERON, H. RONALD. In 1950, 3 sources of budwood of Napoleon sweet cherry that gave differential reactions for virus on Japanese flowering cherry in Oregon were indexed on sour cherry in Wisconsin. One of these had given a negative reaction on both Kwanzan and Shirofugen; one had a positive reaction for virus on Kwanzan and was negative on Shirofugen; and the third had been positive on both flowering cherries. The 1 source that had been positive on both Shirofugen and Kwanzan gave a typical necrotic ring spot reaction on sour cherry, but the other 2 gave no evidence of ring spot. The sour cherry yellows virus was not recovered from any of the 3 sources. In 1951, certain Montmorency trees, used to index each of the 3 sources, developed a severe bark splitting and gumming, primarily on the current season's terminal growth. Symptoms were not expressed until the second year on some trees. All Montmorency trees that showed the bark splitting have continued to express this symptom each year. All 3 of the bark splitting sources produced a dieback and bark splitting when they were graft transmitted to Scout apricot.

Experiments on mint rust control. CAMPBELL, LEO. Experiments on control of mint rust during the uredial stage with fungicide dusts were generally discouraging. However, in small isolated plots zinc undecylenate as 10 per cent dust in 2 applications, the first during the aecial stage and the second during the early uredial stage, in 1952 and 1953 resulted in 90.3 and 57.9 per cent reduction in uredia, respectively. In a small isolated plot in 1953 dinitro amine (Premerge) used as a pre-emerge spray at the rate of 3 lb. actual in 40 gal. of water per acre gave 72.5 per cent reduction in uredia and reduced annual weeds 99.8 per cent. In a 7 acre isolated field, which was likewise treated with Premerge the same year, no rust could be found except near an adjoining oat field where volunteer mint was badly rusted. In 1954 small isolated plots were treated with fungicides and herbicides as pre-emerge sprays at the rate of 3 pounds actual in 40 gal. of water per acre. The percentage reductions in aecial galls, as compared with untreated plots, were as follows: Premerge, 88.8; Karmex, 11.1; Crag 658, 38.8; Manzate, 97.2; Karathane, 88.8; Captan, 100.0; and zinc undecylenate, 95.2 per cent.

Appraisal of bean disease losses in Colombia. CANUTO, CARDONA A., AND ROBERT L. SKILES. Recently attention has been focused in Colombia on the importance of diseases in relation to bean improvement. There are at least 10 different bean-foliage diseases of considerable prevalence in Colombia, of which 7 are common in the Medellín area. They are rust, anthracnose, angular leaf spot, powdery mildew, bacterial common and halo blights, and mosaic. An estimate of the severity of the fungus and bacterial diseases was determined in Medellín by applying various protectants for disease control. Numerous compounds were used in various combinations on several commercial bean varieties over 3 growing seasons. It was found that some bean varieties that had been previously considered poor yielders actually had a very good genetic potential for yield. In the experiments, the check plots of these same varieties yielded only 34–50 per cent of the highest yielding plots. In one of the best commercial varieties available, Algarrobo, the plot with the best treatment outyielded the check by 208 per cent. As a result of these studies, the Colombian bean improvement program is now concentrated more on bringing factors for disease resistance into the commercial varieties to increase yields instead of a combination of genes for resistance and yield.

Some kinetic studies on the reaction of tobacco mosaic virus with formaldehyde. CARTWRIGHT, T. E., AND MAX A. LAUFFER. When tobacco mosaic virus (TMV) reacts with formaldehyde, it loses ability to produce local lesions on *Nicotiana glutinosa* and its anodic electrophoretic mobility increases. When 2 per cent TMV was reacted with 2 per cent formaldehyde at pH 5.3, 6.6, and 7.7, and at 30°, 35°, and 40°C., rate constants for inactivation increased with temperature and were slightly higher at pH 6.6 than at pH 5.3 or 7.7. The reaction was of the first order. Electrophoretic mobility of TMV adsorbed on collodion particles was measured in phosphate buffer of 0.03 ionic strength, by the microscope method. Rate constants for change in mobility increased with both temperature and pH, but there was no evidence of higher reaction rate constants at pH 6.6, as was the case for inactivation. Average mobility of the virus before treatment with formaldehyde was 11.1 cm^2 volt second and after treatment 13.3 cm^2 volt second. Time required for mobility to change depended on conditions of the formaldehyde treatment. The evidence indicates that the reaction which causes loss in infectivity is different from the reaction which causes change in electrophoretic mobility.

Parasitic aggressiveness and its relation to late blight tolerant potato varieties and the survival of the pathogen. CASTRONOVO, ALFONSO, H. DAVID THURSTON, AND CARL J. EIDE. A complex of characters referred to collectively as aggressiveness affects the survival of races of *Phytophthora infestans* and their relative pathogenicity on non-hypersensitive potato varieties. On such varieties differences in infectiveness, incubation period, and sporulation have been found among parasitic races and among different isolates of the same races. Sporangial germination varied so much that differences of genetic origin could not be determined with certainty. The source of sporangia, including small differences in age of the medium on which they were produced, has a definite effect on their germination. Potato varieties differ in susceptibility to infection by races to which they are not hypersensitive, and in the relative abundance with which such races sporulate on them. These host characters, probably related to tolerance or field resistance, are usually assumed to be little affected by differences among races of the pathogen. This assumption may be questioned in the light of demonstrated differences in aggressiveness among isolates of the fungus, and the possibility of the effect of highly aggressive races on such varieties must be reckoned with.

Biotin requirement of Volutella colletotrichoides. CHILTON, JOHN E. Failure of *Volutella colletotrichoides*, a weak parasite of forage legumes, to grow as well on Czapek's agar as on potato-dextrose agar led to vitamin nutrition studies. Screening of 10 vitamins on Czapek's agar showed that growth comparable with that on PDA occurred only when biotin was present. Nutrient solution shake cultures, repeated several times, invariably resulted in added growth when biotin was present. No other vitamins, singly or in combination, induced additional growth. A concentration of 5 μg. biotin per l. of media increased growth as much as 10 times over the no-biotin treatments. Different nitrogen and carbon sources did not alter the biotin requirement. Aspartic acid in the media did not have a biotin sparing effect. Sensitivity level of the fungus was between 0.01 and 0.1 μg. of biotin per l. of media. Little difference in growth increase was apparent between 0.1 and 10.0 μg. per l. biotin concentration.

A slow-growing Aspergillus isolated from stored seeds. CHRISTENSEN, CLYDE M., AND JOHN F. TUITE. Slow-growing strains of Aspergillus, tentatively assigned to the *A. restrictus* series of the *A. glaucus* group species, have been isolated commonly from different lots of wheat and barley stored for 6–12 months at moisture contents of 13.5–14.5 per cent (wet weight basis) and from flax seed stored at a moisture content below 11 per cent. Invasion of the seed by this fungus is associated with decrease in germination

of the seed. The fungus is difficult to isolate from seeds and does not grow well on such common media as potato-dextrose, malt, or Czapek's agar. Of the media tested, the best so far found for isolation of this fungus has been one containing 2 per cent malt extract, 2 per cent agar, and 15–20 per cent sodium chloride. An incubation period of 2–3 weeks at room temperature or 30°C. sometimes is required for the fungus to grow from cultured seeds. Some of the isolates have been strongly antagonistic to other subspecies of the *A. glaucus* group cultured from stored grain.

Honeycombing—a macroscopic symptom of tristeza in Florida. COHEN, MORTIMER, AND L. C. KNORR. More than 90 per cent of the citrus trees found injured to date by tristeza in Florida have shown a pattern of minute holes called "honeycombing" on the inner surface of the bark of the sour orange rootstock just below the bud union. This pattern is found whether the top of the tree is one of the varieties of sweet orange, mandarin, or grapefruit. Honeycombing can be detected by the naked eye, although the use of a hand lens is often helpful. Where honeycoming is typical and appears on the sour orange portion, bark sections from the bud union have been invariably rated positive by the Schneider test for tristeza. However, a range of conditions has been found in bark of sweet orange, rough lemon, sweet lemon, Tahiti lime, and other citrus species, not necessarily at the bud union, that simulate honeycombing to a greater or lesser degree. These may be distinguished from typical tristeza honeycombing by the absence of other histological features of tristeza and by failure to transmit vein clearing symptoms of tristeza to Key lime seedlings when budded from affected trees.

Black spot of helleborus. COTTA, W. J., JR., AND F. L. HOWARD. *Coniothyrium hellebori* C. & M. is a major limiting factor in commercial culture of Christmas Rose (*H. niger*). Conidia incite lesions on above-ground organs during wet periods particularly in fall and spring months as new growth develops. Cardinal temperatures determined for spore germination were: minimum under 4°C., optimum 18 to 24°C., and maximum about 34°C. The temperature range for mycelial growth was the same, except that the optimum was 15°C. Growth occurred on various vegetable media, but not on cellulosic materials such as sugar cane bagasse mulch. Although growth occurred on potato dextrose agar over a range in pH from 2.5 to 9.5, the optimum was between pH 5.5 and 6.5. The spore germtube penetrates directly through leaf epidermis or enters trichomes. Intercellular mycelium causes a dark brown discoloration of invaded tissues. Inoculation of attached leaves on potted plants and excised leaves results in lesion development between 7°C. and 24°C. but not at 4°C. or 30°C. Continuous wetting for 25 hours was required for spore germination and subsequent infection of leaves. Only immature leaves were susceptible and chiefly the lower surface. Leaf wounds do not serve as infection courts. Captan was the most effective fungicide of 7 applied to 1 and 2-year old plants. Phenyl mercury acetate was most toxic to *C. hellebori* conidia and mycelial growth in laboratory tests, but at 1 pt. to 100 gal. of water it injured 1-year old plants.

Factors influencing percentage of seed transmission of the common lettuce mosaic virus. COUCH, HOUSTON B. A considerable difference was found among individual plants of the lettuce variety Bibb in ability to transmit the common lettuce mosaic virus through their seed. Four individual plants of this variety were checked for consistency of seed transmission. The progeny of 3 of these plants varied from their parents' rates of transmission, whereas the fourth remained consistent through 3 generations. The inflorescences of 2 seed-borne mosaic-infected plants were mapped with respect to position and time of floral initiation, and the seed from these different positions on the seed stalk checked for percentage of seed transmission. It was found that percentage of seed transmission was distributed among the various floral heads in a randomized manner, irrespective of topographical relationships, or time and order of floral initiation. Lettuce plants inoculated just before time of flowering produced fewer mosaic-infected seed than those inoculated soon after planting. Plants which became infected after flowering had started did not transmit the virus through their seed.

Pathological anatomy of floral heads of Cheshunt Early Giant lettuce infected with common lettuce mosaic. COUCH, HOUSTON B. A count of 4,684 seedlings, grown from seed harvested from mosaic-infected plants of the variety Cheshunt Early Giant, failed to reveal any seed transmission. It was observed that the first-formed floral heads of infected plants of this variety were killed by the virus. These plants, however, recovered to produce normal seed. The infectivity of the virus in these recovered side shoots, as measured by local lesion production on *Gomphrena globosa* L., was ½ that of younger diseased plants. A study of the pathological anatomy of mosaic-infected floral heads of this variety showed that the procambial and epidermal tissue of the receptacle, and the vascular and epidermal tissue of the peduncle, were especially sensitive to the virus. Complete obliteration of the procambial tissue was quite characteristic, whereas the vessels and laticiferous elements of the peduncle were filled with a gummous exudate. No correlation was found between stage of development and the blighting of the first-formed floral tissue.

Some relationships of carbon disulfide and Trichoderma viride in the control of Armillaria mellea. DARLEY, ELLIS F., AND WESLEY D. WILBUR. Previous laboratory studies demonstrated that sub-lethal dosages of CS_2, while not killing *A. mellea* in roots directly, initiated biological control by stimulating *T. viride* to invade and kill Armillaria after 30 days. Present studies confirm unpublished results of others, namely, that following field fumigation with 2 oz. per 2.25 sq. ft., isolations from infected roots buried at 3 ft. yielded Armillaria the first 2–3 days, were sterile for the next 4–8 days (indicating direct kill), yielded bacteria (non-pathogenic to Armillaria) up to 30–50 days and Trichoderma regularly thereafter. Trichoderma thus appeared to be incidental to control. There appeared to be no direct kill when the dosage was reduced to ½ oz. Armillaria was isolated up to 26 days and Trichoderma was isolated after the 30th day, thus confirming laboratory observations. Trichoderma was stimulated to lethal activity in fumigated soils at standard or reduced dosages only if Armillaria-infected roots were present. Trichoderma did not develop in the field wherein roots were buried 18 days after fumigation or in soils in jars which were fumigated and aerated prior to introduction of roots. Once Trichoderma was stimulated in fumigated soil containing infected roots, its lethal activity continued for at least 3 months.

Species of Ophiostomataceae associated with Engelmann spruce bark beetle. DAVIDSON, ROSS W. A survey of wood-staining fungi associated with *Dendroctonus engelmanni* Hopk. attacking *Picea engelmannii* Parry in Colorado has disclosed the presence of 4 species. A *Leptographium* sp., imperfect fungus (*Grosmannia*), is the most conspicuous and consistent associate of the beetle. This fungus causes conspicuous gray stain in sapwood of attacked trees. *Endoconidiophora coerulescens* Münch is sometimes present in the beetle galleries, causing stain. Two *Ophiostoma* species have been isolated from galleries or from adult beetles. The role of these fungi, especially *Leptographium*, in the life cycle of *D. engelmanni* is not known but possibly they may importantly affect the trees and beetles after attack or later in the successful development of broods. The 4 fungus species from spruce represent all genera (*Ophiostoma, Endoconidiophora,* and *Grosmannia*) of the *Ophiostomataceae. Ophiostoma* is a large miscellaneous group whereas *Endoconidiophora* and *Grosmannia* are smaller natural groups. The groups are most readily recognized by the type of conidia produced. All Ophiostomataceae appear adapted to insect dissemination.

The source and role of phenols in Fusarium wilt symptoms. DAVIS, DAVID, AND A. E. DIMOND. Vascular discoloration in Fusarium infected tomato plants has been attributed to melaninization of free phenols from enzymatically hydro-

lyzed β-glucosides. Free phenols may also be derived from hydrolysis of tannins and lignins or by shunting of phenols to melanin formation at the expense of synthetic reactions. Neither tanninase nor ligninase was detected in the vascular sap of diseased plants. However, diseased plants contain less lignin and fewer lignified cells than healthy plants. In lignin formation, according to Freudenberg, phenols from β-glucoside hydrolysis are oxidized to quinones. The decrease in lignin of diseased plants could arise by shunting phenols to melanin formation at the expense of lignin synthesis. The role of phenols in the wilting mechanism was studied by using mono- and poly-ortho-phenols and their carboxy acid analogues in plants. At 500 p.p.m., catechol, pyrogallol and gallic acid produced vascular discoloration. Only gallic acid caused the wilting and chlorosis typical of Fusarium wilt. Therefore, certain phenols may participate in producing both vascular discoloration and wilting.

The chemotherapeutic activity of unsubstituted heterocyclic compounds. DAVIS, DAVID, AND CHIEN-PEN LO. Unsubstituted heterocyclic compounds were tested as chemotherapeutants on Fusarium wilt of tomato. Neither 5-membered heterocycles nor 5-membered heterocycles condensed with 1 benzene ring were chemotherapeutic. However, 5-membered rings condensed with 2 molecules of benzene markedly reduced disease. Dibenzofuran, dibenzothiophene and fluorene all showed chemotherapeutic activity. The nitrogen analogue, carbazole, although not chemotherapeutic was activated by an alkyl substitution on the nitrogen. The equivalence in biological activity of dibenzofuran, dibenzothiophene, fluorene and alkyl N-substituted carbazoles may arise from their isosteric nature. The contrasting chemotherapeutic potency of carbazole and of alkyl N-substituted carbazoles may be due to the cell permeating qualities of these molecules. Carbazole forms molecular aggregates due to hydrogen bonding, as indicated by its anomalously high melting point. In contrast, the lower melting point alkyl N-substituted carbazoles do not associate, enter the cell more readily, and thus may produce a chemotherapeutic effect. Six-membered heterocycles were not chemotherapeutic even when condensed with 2 benzene rings.

Idaho Bountiful and Golden Gem: curly top- and mosaic-resistant snap beans. DEAN, LESLIE L., AND C. W. HUNGERFORD. Idaho Bountiful and Golden Gem represent the first high quality bush type snap beans resistant to curly top and 2 strains of common bean mosaic. Mosaic resistance in these varieties was derived indirectly from Corbett Refugee; curly top resistance from Burtner Blight Proof. Idaho Bountiful originated from a backcross of Bountiful with a curly top- and mosaic-resistant flat podded hybrid. The plant is upright, approximately 15 in. tall, matures 3–5 days later and is darker green than Bountiful. Pods are flat, being slightly narrower and thinner than Bountiful pods, approximately 5½ in. long, straight, medium green in color, and superior in quality. Pods are entirely stringless with firm medium green flesh. The light brown seed average 85 per oz. Golden Gem, a white-seeded wax bean of exceptionally high quality, was selected from progeny of a cross involving Early Wonder Wax and a curly top- and mosaic-resistant Refugee type hybrid. Plants are low and somewhat spreading. The round wax pods are 4–4½ in. long, straight, 100 per cent No. 1 sieve size, and very uniform. They are stringless without noticeable fiber and have dark, firm flesh. Seeds average 100 per oz.

A tobacco mosaic virus strain highly virulent to tomato. DESJARDINS, P. R., J. M. WALLACE, AND R. J. DRAKE. A yellow strain of tobacco mosaic virus (TMV) that is highly virulent to tomato has been isolated from a virus disease complex in tomato. Isolation was accomplished by inoculation from local lesions produced by the virus complex on leaves of *Nicotiana glutinosa*. The other component of the virus complex was found to be a mild strain of TMV. When tomato plants were simultaneously infected with both strains, the mild strain eventually became predominant and afforded some protection to the plants from the yellow strain in that they continued to grow, flower, and produce fruit. When introduced alone into tomato plants, the isolated yellow strain generally caused death of the plants. Tomato plants infected with an ordinary yellow strain of TMV reacted in a manner similar to those infected with the total virus complex. Pepper plants, if inoculated at a young enough stage, seemed to be more severely affected by the total virus complex than by either of the isolated strains. On leaves of *Gomphrena globosa*, the isolated yellow strain induced the formation of red bordered local lesions which later developed into systemic infection of the leaves. It failed to produce lesions on leaves of bean, variety Early Golden Cluster.

Free amino-acids and carbohydrates in gall and healthy tissues of corn. DEVAY, J. E., AND J. B. ROWELL. Comparative analyses of healthy and gall tissues of corn for free metabolites such as amino-acids and carbohydrates have been made in order to define mechanism of gall initiation as well as nutritional requirements of the dicaryophase of *Ustilago zeae* and gall tissues. Chromatographic analyses of alcoholic extracts of gall and healthy tissues of corn seedlings have shown marked differences in their fructose and glutamine content. Actively growing gall tissues contained more fructose and less glutamine than healthy tissues. No significant differences were found in the concentrations of glucose, maltose, and sucrose in gall and healthy tissues. Similarly, these tissues contained approximately the same concentrations of glutamic acid, aspartic acid, glycine, serine, asparagine, alanine, histidine, arginine, lysine, gamma-amino butyric acid, proline, valine, methionine, cystine, leucine and/or isoleucine, and tyrosine. Whether these differences reflect the metabolic activities of the pathogen and/or the altered physiology of the host cells is being determined by in-vitro studies of gall cells and the dicaryophase of *U. zeae* using media formulated from these analyses. A similar approach may be useful in determining the balance of nutrients which other obligately parasitic fungi require for growth on artificial media. (This research was supported in part by a grant from the U. S. Atomic Energy Commission, Contract No. AT (11-1)-42.)

Pythium root rot of parsley. DE ZEEUW, DONALD J. A species of *Pythium* isolated from parsley near Grand Rapids, Michigan, causes a root rot and loss of vigor, during the cutting season. Yields from sections of fields in which the disease is severe are reduced as much as 50 per cent. Affected plants are stunted and chlorotic, often with a purple discoloration of leaves. Furthermore, they have poorly developed secondary root systems because of cortical sloughing and browning of the smaller roots. Early infection may kill the plant by girdling the taproot at any time after emergence, but post-emergence damping off is usually not severe. Affected plants that survive produce a partial crop of poor quality. Older plants developing extensive tap root lesions during the season die when brought into the greenhouse. Mycelium of both *Pythium* and *Rhizoctonia* is conspicuous in sectioned roots of such plants. Typical symptoms were reproduced in Paramount parsley by transplanting into *Pythium* infested steamed soil. The same symptoms in the greenhouse also developed in plants grown in non-steamed soil from the original source. All controls remained healthy. The pathogen is identified tentatively as *P. debaryanum* Hesse. It appears to be distinct from *P. megalacanthum* de Bary previously reported causing a similar disease. Host range studies of this *Pythium* are being made.

Seasonal variations of free amino acids in healthy and virus-diseased cherry and peach leaves. DIENER, T. O., AND N. O. LAW. Leaf samples were collected from 4 sweet cherry trees (2 healthy, 1 affected with rasp leaf, 1 with mottle leaf), from 2 *Prunus mahaleb* trees (1 healthy, 1 affected with ring spot), and from 8 peach trees (5 healthy, 3 affected with Western-X disease). Sampling began shortly after bud break and continued at 2-week intervals until

leaf fall. Using 2-dimensional paper chromatographic methods, each sample was analyzed for free amino acids. γ-amino butyric acid, alanine, glutamic acid, aspartic acid, and glutamine were found in all samples. Valine, serine, threonine, (iso)leucine, β-alanine, and asparagine were present at all times in peach leaves, in cherry and *P. mahaleb* leaves mainly in spring and late fall with occasional small amounts in midseason. Phenylalanine was absent in late fall samples, glycine appeared only in the latter part of the season, and arginine, histidine and as many as 9 unidentified ninhydrin-reacting compounds were found in early season samples only. Proline, which was found only in spring and late fall in healthy trees, was present during the entire season in peach leaves affected with Western-X disease. Pipecolic acid, which was not present in healthy leaves (except in cherry and peach leaves in early spring) was found during most of the season in leaves affected with Western-X disease.

Control of pear blight by a streptomycin-terramycin mixture. DUNEGAN, JOHN C., JESSE R. KIENHOLZ, ROY A. WILSON, AND WALTER T. MORRIS. At Marysville, California, spray mixtures containing 30, 60, or 100 p.p.m. of streptomycin and 1/10 as much terramycin controlled blight (*Erwinia amylovora*) on Bartlett pear trees without russetting the fruit, when applied 3 or 4 times at intervals of 14 days and 5 or 7 times at intervals of 7 days. The degree of control was inversely related to the concentration of the antibiotic material and the number of applications. In contrast, incidence of blight increased on the check trees as the number of water sprays was increased. Best control was obtained with 7 applications of the highest concentration of the antibiotic material. However, 5 applications of the lowest concentration gave as good control as 3 applications of the highest. The addition of 2 lb. of zineb to each 100 gal. containing 30 p.p.m. of the antibiotic material did not enhance blight control. A standard copper spray material applied 5 or 7 times russetted the fruit and did not control blight so well as the 30 p.p.m. antibiotic material applied the same number of times.

Experiments on the control of bacterial canker of stone fruit trees. ENGLISH, HARLEY, AND C. J. HANSEN. Bacterial canker of stone fruits caused by *Pseudomonas syringae* continues to cause serious damage to California orchards. During the past few years attempts have been made to reduce the severity of this disease by application of protective sprays and by use of certain understocks. Spraying with Bordeaux mixture (8–8–50) at leaf fall and after pruning has yielded erratic results in disease control. In a young Duarte plum orchard, spraying failed to provide protection, whereas, in a mature block of trees, the leaf fall application resulted in a significant decrease in disease severity. Organic mercury compounds have been less effective than copper materials when used as sprays for control of bacterial canker in young apricot trees. Duarte, President, and Santa Rosa plums suffered less from the disease when grown on Lovell peach root than on Marianna plum; similarly, Santa Rosa was less severely damaged on Lovell root than on Myrobalan plum. More cankers developed in Marianna 2624 than in Lovell peach or 2 selections of Myrobalan plum. Beauty and Kelsey plums were highly resistant to the disease, whereas French prune and Duarte, President, and Santa Rosa plum were highly susceptible.

Relation of Phytophthora cryptogea and other Phytophthora species to root rot of alfalfa in California. ERWIN, DONALD C. Phytophthora root rot of alfalfa (*Medicago sativa*) has recently been described by the writer. The causal fungus has been identified as *Phytophthora cryptogea.* This isolate from alfalfa differs from other strains in pathogenicity. It was not pathogenic to roots of aster or tomato (common hosts of *P. cryptogea*). Roots of cotton, sugar beet, carrot, flax, sweet clover, ladino clover, red clover, bean, vetch, cowpea, or trefoil were not rotted by the fungus. The following *Medicago* spp. were susceptible: *M. falcata* (diploid), *M. falcata* (tetraploid), *M. arborea, M. glutinosa,* and *M. lupulina.* Alfalfa was not susceptible to the following *Phytophthora* spp. from other hosts: *P. cryptogea, P. parasitica, P. cinnamomi, P. dreschsleri, P. palmivora,* and 4 unidentified *Phytophthora* isolates. The *P. cryptogea* strain from alfalfa exhibits pathogenicity which may be specific to some species of the genus *Medicago.*

Production and germination of sporangia of the fungus causing sclerophthora disease of sugarcane in Louisiana. FARRAR, L. L., AND R. J. STEIB. A new disease of sugarcane was reported in Louisiana in 1952 and tentatively identified as Sclerospora disease. At this time there was little knowledge concerning the asexual stage. Affected stools of sugarcane were placed in crocks and brought to the greenhouse under conditions of practically 100 per cent humidity. Scrapings from the lower epidermis of diseased leaves revealed an abundance of lemon-shaped sporangia. Attempts were made to germinate sporangia on several agar media, nutrient solutions, rain water, tap water, and distilled water. No germination was observed. In an attempt to determine mode of germination, an all night check on leaf surface scrapings was made. High humidity conditions were initiated at 5 p.m. Immature sporangia were observed at 8 p.m. Mature sporangia were observed after 11 p.m. However, germination was not observed until after 3 a.m. when sporangia were obtained with the protoplasmic contents delimited. Within 15 minutes zoospores were observed to be emitted from the apical end. Further observations revealed several sporangia germinating by the formation of zoospores and several empty sporangia, indicating that germination had already occurred. The number of kidney-shaped zoospores per sporangium ranged from 16 to 24. At present, the fungus is considered to be in the genus, *Sclerophthora.*

A method of predicting wheat streak mosaic epiphytotics. FELLOWS, HURLEY, AND WEBSTER H. SILL, JR. For severe yield reduction to occur, field infection by wheat streak mosaic virus must take place in winter wheat in the fall, although symptoms are seldom seen until spring. By contrast, damage from spring infection of winter wheat has been slight. Since the virus is not inactivated in the plant by low temperatures, a fall-infected plant retains virus through the winter. Dormant plants, therefore, may be transplated to a warm greenhouse (70–80°F.) during fall and winter where symptoms gradually develop in diseased plants. Each fall and winter for 4 years, after the weather became cool enough so that activity of vectors probably was reduced, random samples of winter wheat were gathered at approximately 10-mile intervals in the areas to be surveyed. Each sample contained 12–20 plants taken at random from 4 different areas in a field. In doubtful cases, transfers of the virus were made to healthy plants. Locations of positive and negative samples were charted on a map. By this method it has been possible to predict the location and approximate severity of developing epiphytotics.

The flagella of swarmspores of Phytophthora infestans as viewed with the phase and electron microscopes. FERRIS, VIRGINIA ROGERS, AND H. H. LYON. Examinations of living swarmspores of *P. infestans* (Mont.) de Bary with the phase microscope reveal paddle-like structures on many of the flagellæ. Further information concerning the structure of the flagella was obtained with the electron and phase microscopes. One of the flagella is ciliated, and the other is not. Immediately after the swarmspores emerge from the sporangia, they move about rapidly and no paddle-like structures are visible on the flagella. Later, as the swarmspores begin to swim more slowly, the flagella appear to roll inward from the tip and begin to resemble paddles. Often the flagella may be seen to roll up completely. Before germination occurs, the swarmspore stops its movement and the flagella, still resembling paddles, become detached and float away. Both time and temperature appear to be factors in the rolling up of the flagella and their detachment. Electron micrographs were made of killed and fixed swarm-

spores with the flagella in various stages of rolling up; and a movie was made of the living swarmspores as they appear when the process is viewed with the phase contrast microscope.

Histological study of suscept-pathogen relationships between Solanum demissum derivatives and Phytophthora infestans. FERRIS, VIRGINIA ROGERS, AND L. C. PETERSON. The interactions between leaf tissue of *Solanum demissum* Lindl. derivatives and several races of *Phytophthora infestans* (Mont.) deBary were investigated histologically. Techniques for inoculation and incubation were standardized, and all plants were maintained at 18°C. following inoculation. The histological examinations were made on sectioned material and on cleared whole leaves. In susceptible plants, mycelium was found in tissue distant from the site of penetration within 17–28 hours following inoculation. In resistant plants, short secondary hyphae were often seen between mesophyll cells near the site of penetration, but widely ramifying mycelium was never observed. In most of the resistant plants a necrotic response developed a few hours before such a response was evident in susceptible plants. Differences among the varieties were noted, however, in the rapidity with which a necrotic response developed. Plants duplex for a given resistance gene were inoculated with races pathogenic to plants simplex for the same gene. The lesions on the duplex plants were different in appearance from those found on other plants, whether resistant or susceptible. A susceptible reaction was obtained in the duplex plant when a culture reisolated after serial passages through a plant of the same genotype was used as the inoculum.

A mosaic disease of sweet corn. FINLEY, A. M. A virus which causes mosaic symptoms on sweet corn was isolated from naturally infected plants of the Golden Cross Bantam variety. A comparison of the characteristics of this virus with other viruses which are known to infect sweet corn indicated this to be different from any heretofore described. The first symptoms of disease were small, broken, chlorotic streaks on the terminal leaves. As the virus became systemic, all leaves developed broken or continuous interveinous chlorotic streaks which extended into the leaf sheaths but not the stalks. Affected plants were dwarfed and the yield of marketable corn was small. The virus is mechanically transmissable by leaf rubbing and by hypodermic injection into the stalks. It is not seed borne. The incubation period is 7–21 days, the thermal death point is 45°–50°C. and the maximum dilution of infective plant extract is 1:10,000. Limited host range studies indicated Golden Cross Bantam sweet corn, sweet corn inbred P-39, Gem barley, Lemhi wheat, and wild foxtail (*Setaria viridis*) to be susceptible. Overland oats, orange fodder cane, orchard grass and tobacco were not susceptible.

The genetics of host-parasite interaction in flax rust. FLOR, H. H. A gene-for-gene relationship has been established between rust-resistance in flax and pathogenicity in the rust fungus. By the use of natural and hybrid rust races, 30 lines of flax each apparently possessing a single unique gene for rust reaction have been developed. A revised classification of races of flax rust is based upon the reaction of 18 of these lines. The new differentials were selected for possession of genes conditioning 1) resistance to all North American races, 2) reaction in commercial varieties, and 3) sharp differences in reaction. A routine determination of the reaction of differentials with single rust-conditioning genes establishes the pathogenic genotype of a rust culture. This should facilitate the evaluation of epidemiological data, studies on the role of mutation and hybridization in the origin of new races of the pathogen, studies on the nature of rust resistance, and the development and maintainence of rust-resistant varieties.

Antibiosis as a possible limiting factor of pink root of shallots. FREEMAN, T. E., AND E. C. TIMS. Late in 1952, a field of shallots in LaFourche Parish, La., was examined for pink root. In a portion of the field made up of a silty clay-type soil, severe pink root infection was noted. In an adjacent portion of the field made up of a heavier clay-type soil, pink root was not observed. A study was initiated to determine whether or not antagonistic actinomycetes were a factor in reducing the amount of pink root in the clay soil. To compare the 2 soils in regard to antagonistic actinomycete population, a dual agar layer method was devised to screen large numbers of actinomycetes for ability to suppress the pink root fungus. The results showed that the antgaonistic actinomycete population was higher in the clay-type than in the silty clay-type soil on each of 6 collection dates which covered all 4 seasons of the year. It was also found that certain actinomycetes would materially reduce the severity of pink root in sterilized soil.

The relation between host moisture content and sporulation of Endoconidiophora fagacearum. FRENCH, D. W., AND R. N. CAMPBELL. Moisture contents of stems of healthy and mat-bearing pin oaks were determined in Minnesota in October and November, 1953. On typical, mat-bearing trees the stem could often be divided into 3 zones: the mat-zone where there were fresh, young to mature mats; the zone above this where mats had been produced and were in the process of disintegrating; and the zone below the mat producing area where mats had not been produced but may be subsequently. The moisture contents (wet weight basis) for these 3 zones were 46.6 per cent, 42.8 per cent, and 47.6 per cent respectively for the sapwood and 44.9 per cent, 38.9 per cent, and 47.8 per cent respectively for the outer heartwood. In 3 healthy trees moisture content of sapwood was 42.3 per cent and that of outer heartwood was 47.7 per cent. The sapwood moisture contents showed no differences due to height in the tree. Apparently moisture content of sapwood increases to approximately that of heartwood after the tree wilts. The moisture content of sapwood behind new mats was about 46.6 per cent in contrast to 42.3 per cent for healthy sapwood.

The occurrence, distribution, and spread of stone fruit viruses and a virus-like disorder in Minnesota. FRIDLUND, PAUL R. When budwood of 209 wild trees of *P. pennsylvanica*, *P. serotina*, *P. americana*, and *P. virginiana* were indexed, only 9 trees of *P. virginiana* were found infected with necrotic ring spot. Similarly low percentages of infection were found in 35 varieties of winter-hardy Prunus nursery stock, except for Mount Royal (*Prunus domestica*) which was 100 per cent infected. Other evidence indicates that Mount Royal was introduced into Minnesota as infected budwood. Only 2 questionable cases of natural spread of necrotic ring spot were found in 3 Prunus plantings 8, 5, and 5 years old. X-disease has been observed on wild chokecherry in 31 counties in all parts of Minnesota. Identification was based on inoculation into *P. virginiana*, *P. besseyi*, *P. japonica*, Lovell peach seedlings, and Montmorency on Mahaleb. A necrotic leaf spotting of unknown origin was found in hybrids of *P. besseyi* × *P. salicina*. Surveys of 5287 nursery trees showed 100 per cent of Sapa and Sapalta and 69 per cent of Oka and Opata had symptoms; Compass (*P. besseyi* × *P. hortulana*) and Sioux, Black Beauty and Brooks (*P. besseyi*) had none. Although numerous inoculations were made, no transmissions to *P. tomentosa*, Montmorency, or Lovell seedlings were apparent.

The reaction of Prunus tomentosa seedlings to latent Prunus virus cultures. FRIDLUND, PAUL R. Each of 65 *Prunus* budwood samples was inoculated into 5 potted *P. tomentosa* seedlings at budbreak in the greenhouse. Of these, 49 were determined by investigators furnishing the samples to contain the necrotic ring spot virus, 7 to contain unidentified latent viruses, and 9 to be virus free. The inoculations permitted the separation of these samples into 6 groups: 1) 40 produced similar symptoms on *P. tomentosa* seedlings and symptoms on Montmorency cherry described as those of necrotic ring spot; 2) 8 caused symptoms on *P. tomentosa* similar as a group but distinct from group 1, and probably not those of necrotic ring spot; 3) 5 produced no symptoms on *P. tomentosa*, but were reported to have-

produced symptoms of necrotic ring spot or another latent virus on other index hosts; 4) 3 produced symptoms on *P. tomentosa* distinct from groups 1 and 2 and variable symptoms on other index hosts; 5) 1 produced symptoms on *P. tomentosa* but none on peach, Kwanzan, Shirofugan and questionable on Montmorency; 6) 7 samples produced no symptoms on *P. tomentosa* or any other index host. These results are evidence that *P. tomentosa* seedlings are satisfactory for the detection and identification of the necrotic ring spot virus.

Asexual spore production in Diaporthe phaseolorum var. batatatis. FROSHEISER, F. I., AND M. F. KERNKAMP. Recent workers have indicated that ascospores are the source of inoculum for the spread of stem canker on soybeans, caused by *Diaporthe phaseolorum* var. *batatatis*. No pycnidial formation was observed by the writers in a year's study of the organism in numerous cultures on potato-dextrose agar or on naturally or artifically infested soybean stems in moist chambers, although perithecia were formed abundantly. Recently perithecia formed in abundance on autoclaved stems of 15 crop plants and weeds inoculated with ascospores. Pycnidia with conidia were observed on all the material except soybean, corn, and 3 species of weeds. Monoconidial isolates from autoclaved white sweet clover and wheat stems produced the usual numbers of perithecia on potato-dextrose agar. When mycelium from these cultures was transferred to water agar containing finely-ground sweet clover stems and leaves, pycnidia were again produced in abundance. Blackhawk soybeans were inoculated in the greenhouse with conidia from cultures that were isolated from sweet clover; the organism was reisolated from those plants. These results indicate that the asexual spore stage may be more important in the spread of this disease in the field than has been suspected.

Pathogenicity of fungi isolated from diseased red clover roots in Wisconsin. FULTON, N. D., AND E. W. HANSON. Forty-nine fungus isolates were tested for pathogenicity on the roots and crowns of red clover plants ranging in age from 6 weeks to 6 months. Included were *Fusarium oxysporum, F. solani, F. roseum, F. moniliforme, Rhizoctonia* spp., *Phoma* spp., *Gliocladium roseum, Pythium* spp., the blackpatch fungus, and several others. Tests were made in the greenhouse in white silica sand, in steamed and fumigated soils, and in 5 naturally-infested field soils at controlled temperatures ranging from 16–28°C. None of the isolates was more than weakly pathogenic under optimum growing conditions for the clover. However, the disease level increased considerably in most cases when the plants were weakened by various methods. Clipping and removing the foliage periodically proved to be an effective technique for increasing susceptibility. The number of clippings required to obtain a given level of disease was influenced by temperature, age of plants, and other factors. More time was required to weaken plants at low than at high temperatures. Also, older plants recovered less rapidly after clipping than younger plants. Certain isolates of *F. oxysporum* and *F. solani* were the most pathogenic of the fungi tested. (Cooperative investigations between the Wisconsin Agricultural Experiment Station and the Field Crops Research Branch of the U. S. Department of Agriculture.)

Studies on inactivation of the strawberry Type 2 virus in vivo. FULTON, R. H. Type 2 virus-infected Robinson strawberry plants were grown in vermiculite in 4-in. pots. They were defoliated and placed in forced-air Cenco incubators maintained at 34°, 36°, 38°, 40° and 42°C. for 4, 6 and 8 days. Surviving plants were indexed to *Fragaria vesca* to ascertain effect of individual treatments on the virus. Type 2 virus exhibited a thermal inactivation range from 36° to 40°C. Lowest temperature for inactivation was 36°C. for 8 days. Virus inactivation was most assured at 38°C. for 8 days. The 40°C. treatment was effective for 4 and 6 day periods, whereas all plants died with 8 day exposure. At 42°C. all plants succumbed. Chemical inactivation studies were made on Type 2 virus-infected Robinson plants growing in sand in 4-in. pots. Calcium sulfate, zinc chloride, and zinc sulfate were used at concentrations of 1 and 2 per cent. Individual treatments consisted of 50 ml. of a given chemical solution applied every 4 days for 40 days. Subsequent indexing of test plants revealed that Type 2 virus was inactivated by 2 per cent zinc chloride and zinc sulfate. Actidione, aureomycin hydrochloride, neomycin, and streptomycin sulfate were used at 250 p.p.m. and 500 p.p.m. with negative results.

Inheritance of resistance to tomato late blight. GALLEGLY, M. E., AND M. E. MARVEL. Two types of resistance to tomato race 1 of *Phytophthora infestans* were found. The first, controlled by a dominant gene, occurs in North Carolina cherry tomato (W. Va. #36) and PI 108245 (W. Va. #106). F_1 progeny of #36 and #106, crossed with Marglobe, Rutgers, Wisconsin "55", and Southland varieties, were resistant when 4 weeks old. F_2 progeny segregated 3 resistant to 1 susceptible and 2 backcrosses segregated 1:1. The second type occurs in W. Va. #19 (Fla. P_1), W. Va. #731 (breeding line from P_1), Southland, and Wisconsin "55" varieties and apparently is polygenic. When Marglobe was 100 per cent susceptible, Southland was 8 per cent resistant, 10 per cent intermediate, and 82 per cent susceptible, and #731 was 46 per cent resistant, 36 per cent intermediate, and 18 per cent susceptible. Marglobe $\times$ #731 F_1 progeny were 22 per cent intermediate and 78 per cent susceptible, while those of Southland $\times$ #731 were 28 per cent resistant, 33 per cent intermediate, and 39 per cent susceptible. Marglobe $\times$ #731 F_2 plants segregated 10 per cent resistant, 24 per cent intermediate, and 66 per cent susceptible; those of Southland $\times$ #731 segregated 15 per cent resistant, 60 per cent intermediate, and 25 per cent susceptible. F_3 progeny from resistant F_2 plants were uniformly resistant.

Bacterial streak, a new disease of Narcissus. GOULD, CHARLES J. An unusual collapse of Narcissus stems (variety King Alfred) was observed by inspectors of the Washington State Department of Agriculture in a commercial field in the spring of 1954. About 2 per cent of the plants were infected in a stock that had not been dug for 2 years. Specimens submitted for identification yielded a bacterium. Hypodermic inoculations into the host have reproduced typical symptoms. Field inoculations of the stem bases of 100 plants resulted in collapse of 87 per cent within 7 days. Nine per cent more were infected but failed to collapse. An indistinct grayish-pink discoloration of the base of the stems was evident within 2 days following inoculation. After 4 days the discoloration was very pronounced, varying from pink to brown, often with a violet tint, and from a streak to a general decay. The infected area extended as much as 15 in. after 7 days and was associated with a yellowing of the entire stem. The collapse of the discolored area accompanied by an inward rolling of the marginal tissue was a marked characteristic of the streak type of reaction. Natural leaf infection has not yet been observed but was obtained by hypodermic inoculation, resulting in a brown streak.

Soil treatment tests for the control of Sclerotium rolfsii in bulbous iris. GOULD, CHARLES J. Many western Washington bulb fields have become infested with *Sclerotium rolfsii* (delphinii biotype), which causes a crown rot of bulbous iris. In searching for an effective soil treatment material the rate of growth of the fungus was measured on potato dextrose agar in which the fungicides were mixed, usually at rates of 3 to 100 p.p.m. In such tests in 1952 Mathieson's chlorinated nitrobenzenes 273, 1194, 1197, and PCNB (pentachloronitrobenzene) appeared more promising than 276 and 278, but only PCNB effectively controlled the fungus outdoors in artifically infested soil contained in large concrete sewer tiles. Crag 5400, Penn Salt's NP-1083, and Mathieson's 1589, 1439, and 1440 appeared more promising in 1953 laboratory tests than did Anticarie, captan, PCNB, Mathieson's 1197, Penn Salt's NP-1282, Stauffer's N-244, and N-521. However, in greenhouse tests with artifically infested soil, PCNB gave best results. Shell 51-P-162 (10, 30, and 50 p.p.m.) gave inadequate

control in field tests with naturally infested soil, whereas Shell D-D (dichloropropene-dichloropropane) (68 gal. per acre) and CBP-55 (chlorobromopropene) (30 gal. per acre) increased the loss from crown rot, perhaps through elimination of antagonistic organisms. PCNB rototilled into the soil at 200 and 300 lb. (active) per acre appreciably reduced the disease.

Distribution of races of Phytophthora infestans in Canada. GRAHAM, K. M. During 1952 and 1953, some 75 isolates of *P. infestans* (Mont.) deBary were obtained from diseased potato tubers and tomato fruits received from the 10 provinces of Canada. Only Races 0 and 4 (international classification) were isolated from commercial potato varieties lacking genes for resistance. On varieties such as Canso, Keswick, Placid, and Kennebec, carrying 1 or more genes for resistance, Races 1, 1.2, 1.4, and 1.2.4 were found. No clear-cut distinction between "potato" and "tomato" races was apparent. Races 0 and 4 were both isolated from tomatoes, while a third race, tentatively designated 1(T), was obtained from Snowflake potato, the tomato variety Rutgers, and from a cherry tomato carrying a single gene for resistance to Races 0, 4, and to all the other races hitherto obtained from potato. Race 1(T) behaved like Race 0 on the potato differentials, whereas isolates of Races 0, 1, 4, 1.2, 1.4, and 1.2.4. all attacked Stokesdale tomato. There was some evidence that a few of the original isolates consisted of mixtures of races. After being purified by means of a single-zoospore technique, all races hitherto tested have behaved consistently.

Nuclear behavior in Phytophthora infestans (Mont.) de Bary. GRAHAM, K. M. The ability of the sporangia, sporangiophores, and hyphae to take on the stain seemed to depend upon their physiological age. The most satisfactory fungus material for this study was that which was collected from its natural hosts when it was beginning to sporulate. A modified Feulgen technique was used. Nuclei which seemed just about to divide, or which had apparently divided, were smaller and more easily stainable than resting nuclei. Vegatative hyphae and sporangia are multinucleate. Single nuclei were observed in some very young sporangia. Others contained as many as 4 nuclei. On occasion, well-defined mitotic anaphase figures were visible. Chromosomes were seen though they were somewhat clumped. The larger sporangia contained an indefinite number of nuclei. The numerous zoospores seen were all uni-nucleate. These observations indicate that a single nucleus migrates into the sporangium at the time of its initiation, and that the zoospores of a given sporangium contain sister nuclei. A culture from a single zoospore or a single sporangium ought therefore to be genetically pure and may be used for the establishment of a pure culture of any given race.

The role of nitrites in the wilt response induced by Verticillium albo-atrum R. & B. GREEN, RALPH J., JR. Bonny Best tomato seedlings approximately 10 in. tall were inoculated by the root-dip method, planted in washed quartz sand, and maintained at a constant temperature and relative humidity. Symptoms were observed within 5–7 days. At intervals, tracheal fluids were collected from decapitated plants by negative pressures equivalent to 0.6 atmosphere. Nitrate and nitrite levels determined colorimetrically demonstrated a gradual decline in nitrate levels in infected plants to a low of 24 p.p.m. after 45 days and a corresponding increase in the nitrite concentrations, to a maximum of 62 p.p.m. However, there was no significant difference in the nitrite levels in the tracheal sap from normal and infected plants until after 20 days. Slightly etiolated tomato seedlings, with roots excised, were placed in samples of tracheal sap from normal and infected plants. Test cuttings were transferred to tap water and observed 4 days for symptom manifestations. No differences were noted in transpirational rates of seedlings in tracheal fluids from normal and infected plants and no symptoms were expressed after 7 days. The results of this investigation failed to support the proposal by earlier investigators that the accumulation of nitrites is a significant factor in the wilt phenomenon induced by *V. albo-atrum.*

Effect of soil fumigation and planting stock on Verticillium wilt of peppermint. GREEN, RALPH J., JR. AND JOHN V. LUCK. Preliminary screening of the vapor phase of more than 30 potential soil fumigants against the mycelial, spore, and sclerotial stages of *V. albo-atrum* revealed the 2 most effective materials to be CBP-55 (chlorobromopropene) and Cpd 162 (pentachlorodiene). Tests in muck soil under greenhouse conditions showed that concentrations 2× to 3× above that recommended for mineral type soils were necessary to give comparable results. Field plots incorporated 3 levels of fumigant application and 3 sources of planting stocks. The results, based on percentage infection and yields in green top weight, demonstrated that CBP-55, at rates equivalent to 80 gal./acre, gave highest yields and lowest infection percentages. In addition, survival was poor in heat-treated rhizomes and there was no significant difference in infection levels between field-dug and known disease-free planting stock.

The apparent graft transmission of a factor causing tuber formation in potatoes. GREGORY, LUIS E. The effect of photoperiod and temperature on the growth and development of virus free potatoes of the variety Kennebec was studied at the Earhart Plant Research Laboratory at Cal Tech. High tuber yield was obtained when the plants were grown at a day temperature of 20°C. with 8 hours of natural light and a night temperature of 14°C. When grown at a day temperature of 26°C. with 8 hours of natural light, a night temperature of 20°C. plus 8 additional hours of 1000 f.c. maximum intensity artificial light, no tubers were produced. Cuttings removed from plants induced to form tubers will also form tubers from the axillary bud at the base of the cutting, whereas no tubers are formed from non-induced cuttings, but instead the bud will grow into leafy shoot. An induced stem grafted to a non-induced stem, and the grafted stock and scion maintained under conditions which prevents tuber formation, will still cause the axillary bud at the base of the stock to differentiate a tuber in about 7 days.

Fusarium wilt—a major factor in the decline and replant problem of asparagus in California. GROGAN, R. G., AND KENNETH KIMBLE. Asparagus plantings on land not previously used for asparagus usually reach peak production in about 8 years. The profitable duration of an asparagus planting is usually 10–15 years. After reaching peak production, there is a gradual decline in spear size and stand until the planting is no longer profitable. It is difficult and often impossible to reestablish a profitable asparagus planting on old asparagus land. Below-ground parts of declined plants show considerable destruction of storage and feeder roots, rustlike flecking of spears, and some reddish-brown discoloration of the vascular system. Isolations from discolored tissues yielded a variety of fungus organisms, the most prevalent and pathogenic being *Fusarium oxysporum* f. *asparagi.* This fungus occurs to a greater or less degree in all plantings which have been examined. Washings from commercial seed samples were used to inoculate seedlings. Results demonstrated that the organism is frequently seed-borne as a surface contaminant. This work strongly indicates that a major factor in the decline and replant problem of asparagus in California is Fusarium wilt. A seedling test has been developed in an attempt to find resistance to this disease.

The false stripe V, a necrogenous reaction delimiting infection. HAGBORG, W. A. F. Natural infection in false stripe (stripe-mosaic) of barley is characterized by a necrotic, somewhat irregular, dark-brown stripe that sometimes takes the form of a "V" or an inverted "V". The leaf area on the distal side of the stripe remains a healthy green color, while the area on the proximal side develops a mosaic pattern of chlorosis. In inoculation experiments, this reaction was produced at will in the secondary leaf of Plush barley. The method was to inoculate the primary

leaf before the secondary leaf had begun to emerge from the leaf roll. The appearance of the striping suggested that it was a necrogenic reaction resulting in delimitation of the infected area from the noninfected. Support for this hypothesis was obtained when separate inoculations were made from the areas separated by the stripe in 15 different leaves. In every instance, juice from the proximal area was infective and from the distal area noninfective. When older plants were used, either a reduced area or none of the secondary leaf was infected, although the tertiary leaf was heavily infected. It is suggested that local resistance to systemic infection in this disease is a function of aging of the tissues.

Screening pea varieties for reaction to the red clover vein-mosaic virus. HAGEDORN, D. J. A search for resistant varieties or strains of pea (*Pisum sativum* L.) to the stunt disease incited by the red clover vein-mosaic virus pointed out the need for an improved method of plant inoculation because the rubbing method was so slow and tedious. To meet this need an air-pressure inoculation technique was devised which enabled more rapid screening of pea varieties for reaction to the virus. This technique involved blowing infective plant sap through an atomizer using 20–24 lb. air pressure. The atomizer was held within 6 in. of the plant and all above-ground portions of the plant were "spray inoculated." Use of 600 grit carborundum powder mixed with the inoculum, ½ teaspoon per 100 cc., appeared to be preferable to previous dusting of the plants with carborundum powder. Pea seedlings with at least 2 pairs of fully-expanded leaves were at an optimum growth stage for inoculuation. Two hundred fifteen varieties or strains of peas, including commercial varieties, collection strains, and plant introduction accessions have been screened successfully using this technique. In 2 trials, only 3 pea strains have failed to develop pea stunt symptoms.

Inheritance of pathogenicity in Ustilago avenae. HALISKY, P. M. The genetics of pathogenicity was studied in a cross between races A-5 and A-6 of *Ustilago avenae.* (Pers.) Rostr. These races differ by the respective resistant and susceptible reactions of the variety Monarch. The pathogenic homozygosity of each race was established by testing inbred lines on 6 host varieties. Each inbred line was essentially identical in pathogenicity with its parent race. The pathogenicity expressed by the F_1 hybrids in an A-5 by A-6 cross suggested incomplete dominance of the A-6 reaction. This was indicated by low infection percentages (0.9 to 8.9) on Monarch by all 8 inter-racial combinations in contrast to the complete absence of infection by A-5 and high infection percentages (50.0 to 73.3) by A-6. In another cross between races A-5 and A-7 the expression of a nonsporulating type of infection on the variety Camas by A-7 was found to be heritable. Six of the combinations between these races produced this type of infection (0.5 to 2.9 per cent) suggesting incomplete dominance of the A-7 reaction. The infected plants were further characterized by extreme reduction in height. Studies on segregation and recombination of factors governing pathogenicity and infection type are incomplete.

Effects of 5 species of Pythium on 2–8 weeks old forage legumes. HALPIN, J. E. Sterile seedlings of alfalfa, Ladino clover, red clover, and sweetclover were inoculated at 2, 4, 6, and 8 weeks of age with pure cultures of *Pythium debaryanum, P. irregulare, P. paroecandrum, P. splendens,* and *P. ultimum.* The seedlings were grown in white silica sand at 68–75°F., and watered with a modified Hoagland solution. Inoculum was added to the sand without disturbing the roots. Each test included 4 replicates and was repeated. Seedlings were lifted, washed, and examined 15 days after inoculation. Whereas all isolates used were known to incite seed rotting and pre- and post-emergence damping-off of these hosts, when incorporated into the sand at the time of seeding, none of them had any effect on stand or caused any apparent root discoloration on plants 2 weeks old or older. (Cooperative investigation between the Wisconsin Agricultural Experiment Station and the Field Crops Research Branch of the U. S. Department of Agriculture.)

Physiologic races of Ustilago avenae and U. kolleri in Kansas. HANSING, E. D. The standard differential varieties Anthony, Black Diamond, Victory, Gothland, Monarch, Black Mesdag, Fulghum, Camas, Nicol, and Lelina were inoculated with 64 collections of *Ustilago avenae* and 38 collections of *U. kolleri* to determine the number, prevalence, and distribution of physiologic races of these fungi. Three new distinct Fulghum and 2 new non-Fulghum races of *U. avenae* were established and designated as A-16 to A-20. Fulghum, Black Diamond, and Gothland were susceptible to the new Fulghum races. A-16 was characterized by the additional susceptibility of Anthony and Victory, A-17 by the susceptibility of Monarch, and A-18 by none additional. For the new non-Fulghum races of *U. avenae* Anthony, Black Diamond, Victory, Gothland, and Monarch were susceptible or intermediate in reaction. In addition, Nicol was intermediate in reaction to A-19, and Lelina was susceptible to A-20. Other races of *U. avenae* described were A-1, A-5, and A-12. A new race of *U. kolleri* was characterized by the susceptibility or intermediate reaction of Fulghum, Anthony, Black Diamond, and Victory and designated as K-8. K-4 also was identified. The Fulghum and Victoria (Lelina) races were more common in southern Kansas whereas the other races were generally distributed.

Effects of source and method of applying inoculum on virus infection in clovers. HANSON, E. W., AND D. J. HAGEDORN. Alsike clover, crimson clover, red clover, sweetclover, and garden pea were compared as sources of inoculum of alfalfa mosaic virus, red clover vein-mosaic virus, and yellow bean mosaic virus. Infectivity of inoculum from the various sources was determined on healthy plants of the 4 above-mentioned clover species. With few exceptions, inoculum from all the hosts yielded excellent infection. Alfalfa mosaic virus from red clover did not give quite as good infection, particularly when tested on red clover, as did inoculum from the other sources. Red clover vein-mosaic virus from alsike clover was inferior to other sources in tests on red clover but nearly as good on crimson and sweetclover. Best sources of inoculum of the yellow bean mosaic virus were crimson clover and garden pea. Five methods of applying inoculum of the 3 viruses to the clovers were compared. These were: atomizing leaves, petioles, and crowns; rubbing leaves with cloth pad, glass spatula, or fingers; and hypodermic injection of stems and crowns. In all but the latter method, carborundum was applied to foliage with a powder blower prior to inoculation. The rubbing methods were about equally satisfactory and much superior to the other methods studied. (Cooperative investigations between the Wisconsin Agricultural Experiment Station and the Field Crops Research Branch of the U. S. Department of Agriculture.)

Comparative effect of different stem rust infection types on the yield of Lee wheat. HART, HELEN, AND E. B. HAYDEN. Triplicate randomized 18-ft. rows of Lee wheat were inoculated in the boot stage with race 11 and race 15B of *Puccinia graminis tritici* to determine the relative effect of different rust infection types on yield. Adult plants of Lee are resistant to race 11 (infection type 1) and susceptible to race 15B (infection type 4). Plants in each row were moistened with water, inoculated by cyclone duster with a mixture of talc and urediospores of one rust race, then covered with transluscent plastic from 8 p.m. until 8:30 a.m. the next morning. Uniform, moderately heavy infection resulted; an initial severity of 30–35 per cent increased to 50–60 per cent at maturity. Natural infection was late in control rows and the severity at maturity was 30–40 per cent. If the average yield of the control rows (273 gm.) is taken as a base, rows inoculated with race 11 yielded 85 per cent (232 gm.) of the control, and rows inoculated with race 15B yielded 60 per cent (163 gm.) of the control. A quantitative measurement of the effect of infection type on yield was made possible by the

use of an inoculation and incubation technique which resulted in early and uniformly heavy infection before natural infection obscured differences.

Direct germination of the sporangia of Phytophthora infestans. HARVEY, H. T. At 25°C. in water, 25 per cent of the sporangia of *Phytophthora infestans* from Colma, California, germinated directly by germ tubes whereas less than 10 per cent germinated by zoospores. At 13°C. corresponding values were 5 per cent direct and 90–100 per cent indirect germination. At 25°C. in water, about 50 per cent of the sporangia of a culture from Pennsylvania germinated directly, while none produced zoospores. At 13°C. corresponding values were about 20 per cent direct and about 10 per cent indirect germination. Potato leaves with the Colma isolate sporulating on them were exposed to air at less than 50 per cent relative humidity and about 25°C. for 1–5 hours. When sporangia from these leaves were dusted on slides and placed in moist chambers at 13°C., direct germination was about 20 per cent. For sporangia not exposed to dry air 0–5 per cent germinated directly. Indirect germination did not occur in either case. Concentrations of 20 per cent ethyl or methyl alcohol induced as much as 21 per cent direct germination. Concentrations of about 10 per cent leaf extract from various plants induced as much as 81 per cent direct germination. The substance in leaves favoring direct germination is soluble in water, heat stable, and dialyzable.

Forecasting decay in stored California table grapes. HARVEY, JOHN M. California table grapes are held in cold storage as long as 6 months before they are marketed. Decay in experimental lots of stored grapes of the Emperor variety has been successfully forecast for the last 2 years by use of a method based on the measurement of incipient infections in grapes at harvest. Representative samples of each lot of grapes were collected immediately before storage, surface-sterilized by fumigating with sulfur dioxide, and incubated under aseptic, moist conditions at room temperature for about 10 days. A high positive correlation was found between the percentage of infected grapes in the samples and the amount of decay that subsequently developed in the corresponding lots in storage. In commercial use this method of forecasting decay may serve as a useful guide to the length of time each lot of grapes may be safely stored.

Biotypes of Puccinia graminis tritici virulent on Khapli emmer. HAYDEN, E. B., D. H. SMITH, AND G. C. PAPAVIZAS. Khapli emmer has been generally resistant to *Puccinia graminis tritici* in the United States for more than 40 years. Race 11 was isolated from medium size uredia on Khapli with 60 per cent stem rust in the 1952 nursery at St. Paul, Minnesota. It was compared with several isolates furnished by the Federal Rust Laboratory: race 11 from other sources, the closely related race 32, race 15B (especially an isolate from Oklahoma), and race 14. The new isolate of race 11 and an Iowa isolate of race 32 produced infection types 2 to 3+ cn on Khapli seedlings at 80–85°F., whereas types 0; to 2 were produced by other isolates of these races. The Oklahoma 15B produced types 0;, 1, and 3+, whereas normal 15B produced 0; to 1. An isolate of race 14 from France produced types 2 to 3++c, whereas normal 14 produced type 0; on Khapli seedlings. Adult plants of Khapli were moderately susceptible to the new isolate of race 11 and to the Iowa isolate of race 32. These isolates were compared with a composite of isolates of race 15B on a number of breeding lines deriving rust resistance from Khapli emmer. The isolates of races 11 and 32 were less virulent than the composite of isolates of race 15B.

Strain relationships of the ring spot virus on cherry, peach, and cucumber. HEINIS, J. L. AND J. A. MILBRATH. Twenty-three different stone fruit trees were indexed for ring spot virus on peach, Bing sweet cherry and Kwanzan and Shiro-fugen flowering cherry. Twenty-two of these caused virus responses of varying severity that could be classified as very mild, mild, severe, and very severe. Reactions were classified as very mild if there was any indication of virus infection on any of the test plants, mild if a noticeable leaf symptom developed, severe if the leaves were seriously affected, and very severe if considerable killing and die-back occurred. By using very young leaves and flower petals from peach, sweet, sour, or mahaleb cherry as sources of inoculum, a virus was transmitted to cucumber from 20 of the 22 sources having ring spot virus. The cucumber showed great variation in reaction to these inoculations, which would indicate strain variation. When reactions on cucumber that were classified as mild, severe and very severe were compared with severity of reaction on the stone fruit hosts, a close correlation was noted. This correlation strongly supports the theory that the virus transmitted to cucumber is the ring spot virus of stone fruits.

Virus infectivity and host tissue growth on synthetic media at different temperatures and acidities. HILDEBRANDT, ALBERT C., A. J. RIKER, AND JEAN L. WATERTOR. *Nicotiana tabacum* tissue of crown gall origin was studied because it contained tobacco mosaic virus and had grown in vitro 4 years on synthetic media. Growth was measured as wet weight, and virus activity as numbers of local lesions. Over 6,000 cultures were employed. Cultures were incubated at temperatures between 4° and 42°C. Cultures grew excellently from 20° to 36°, well at 16° and 37°, and poorly or not at all at 4°, 8°, 38°, 39° and 42°C. Local lesion production was greatest from cultures at 24°, 26°, and 28°C., least at 36° or 37°, and intermediate at the other temperatures. Media with acidities from pH 2.0 to 11.5 were tested. Growth occurred between pH 4.5 and 8.5. The range for optimum growth was pH 5.0 to 6.5. No growth appeared below pH 4.0 or above pH 9.0. Six weekly assays showed progressively more local lesions per unit weight from cultures on media with progressively increasing pH values from pH 2.5 to 8.7. Most local lesions commonly developed from cultures incubated at pH 8.3 to 8.4, with few or no local lesions from cultures on media with pH values higher than pH 9.3.

The biochemical action of N-trichloromethylthiotetrahydrophthalimide (Captan). HOCHSTEIN, PAUL E., CARROLL E. COX, AND HUGH D. SISLER. Preliminary investigations on inhibition of growth of conidia of *Fusarium roseum* Lk. by N-trichloromethylthiotetrahydrophthalimide (captan) indicated that carbohydrate degradations were being affected by the compound. Chromatographic studies showed that pyruvic acid accumulated in captan-treated conidia utilizing glucose under both aerobic and anaerobic conditions. It was found that captan strongly inhibited the decarboxylation of pyruvic acid by a crude brewers' yeast carboxylase preparation. This inhibition was reversed by addition of small amounts of thiamine pyrophosphate (cocarboxylase) even after incubation of the enzyme with captan prior to the addition of coenzyme. No in vitro reaction between captan and thiamine pyrophosphate could be demonstrated, rather the inhibition was shown to be of the coenzyme-competitive type. It is suggested that captan affects cellular metabolism by blocking key decarboxylation reactions in which thiamine pyrophosphate functions as the coenzyme.

Preventive and curative effects of thiouracil treatments in mosaic-hypersensitive tobacco. HOLMES, F. O. Thiouracil treatment has not been reported to prevent or cure the classical chlorotic-mottling type of tobacco-mosaic disease in mottling-type tobacco varieties but only to delay its onset. Recent experiments showed that treatment of mosaic-hypersensitive tobacco could be more effective than treatment of mottling-type lines. Application of 5 mg. of thiouracil to each plant daily, in 0.01 per cent aqueous solution applied as a soil drench, enhanced resistance substantially when continued for periods of 4–12 days. Pretreatments reduced the number of primary lesions in inoculated leaves and prevented systemic disease. In these experiments, inoculations were heavy enough to cause systemic spread of virus, with resultant top necrosis and severe stunting or death, in most untreated control plants. If treatments were

delayed until just after primary lesions appeared, almost all plants receiving 5 mg. of thiouracil daily for 4 days showed no further disease. Treated plants grew to maturity, blossoming and setting seeds, without relapse. Cure of disease appeared complete.

Natural hybridization between common and dwarf bunt as related to the problem of delimitation of species of Tilletia occurring on wheat. HOLTON, C. S. Experimental demonstration of hybridization between species and races of the bunt fungi (*Tilletia caries* and *T. foetida*) is the basis for speculation that natural hybridization among these entities occurs in the field. Recorded evidence of natural hybridization in the common bunt type is based on the presence of "hybrid-type" spores in field collections. Evidence of natural hybridization between the common and dwarf bunt types is now available from studies of material found in a field of Elgin wheat infested with both types. Wide variation in certain characters which sometimes distinguish between common and dwarf bunt indicated segregation and recombination of genetic factors governing those characters. Plant height, for example, exhibited a complete series of intergrading types between the extremes. Intermediate types were also exhibited by such characters as size and shape of spore balls, spore morphology, and rate of spore germination. Similar ranges in variability of these characteristics have been observed in populations of experimentally produced hybrids between dwarf and common bunt. The natural occurrence of hybrid and segregating populations in the species of *Tilletia* on wheat has obvious implications in relation to the problem of species delimitation in this group.

Plant parasitic nematodes in relation to strawberry culture in Louisiana. HORN, N. L., AND W. J. MARTIN. A preliminary survey of the nematode population in strawberry soils of Louisiana indicated the common occurrence of large populations of *Tylenchorhynchus claytoni, Tylenchorhynchus* sp. and *Helicotylenchus* sp., and a more limited occurrence of *Pratylenchus* sp., *Trichodorus* sp. and *Meloidogyne hapla.* Experiments showed that *T. claytoni, Helicotylenchus* sp. and *M. hapla* propagated readily on roots of the Klonmore variety growing in steam-sterilized soil, with severe root damage resulting from *M. hapla* only. Soil fumigation experiments with ethylene dibromide in commercial fields of the Klonmore variety resulted in increased growth and yield in soils infested with *M. hapla.* Although increased growth appeared evident in one test where the soil was heavily infested with *T. claytoni,* no response in yield was obtained as a result of the fumigation. Populations of all parasitic species were reduced greatly in the fumigated plots and remained at low levels during the entire production period.

Factors affecting the disease cycle of peppermint rust. HORNER, C. E. Mint rust (*Puccinia menthae* Pers.) overwinters on peppermint in Oregon as teliospores only. Teliospores produced during November reached maximum germinability in January and continued to germinate for more than 108 days. Infection of young peppermint shoots by basidiospores occurs as early as December in the field, but development of spermogonia and aecia is greatly retarded by low temperature. Similarly, field infections caused by aeciospores during February and early March require an incubation period 3 times as long as greenhouse infections. Aeciospore viability was reduced from 76 to 6 per cent when field temperatures dropped below freezing. Viability of urediospores on living leaves exposed to direct solar radiation was reduced from 63 to 36, 23, and 12 per cent after 2, 4, and 8 days exposure, respectively. Viability of urediospores protected from direct solar radiation was not affected. Rust mycelium and associated host cells were killed by exposure for 4 hours to temperatures of 90°F. and above, even at high relative humidities. Losses in oil production are associated with excessive desiccation of infected host tissue. Losses from moderate infection with high temperature and low humidity were as great as from severe infection under moderate temperature and humidity.

Chemical control of Sclerotinia rot of Ladino clover. HOUSTON, BYRON R., JOHN W. OSWALD, AND MAURICE L. PETERSON. Sclerotinia rot (*Sclerotinia sclerotiorum* Eriks.) has become increasingly severe in Ladino white clover during the past 3 years in California. Blocks in a uniformly infested field were treated in January, 1953, using 9 fungicides in replication. The only material which suppressed activity of the fungus was penta-chloronitro-benzene (Mathieson Chemical #275) used at 9 or 18 lb. of active material per acre either as a spray or dust. The fungus was completely checked and did not again become active during the normal period of its activity to April 1, 1953. Treated areas rapidly recovered and showed normal growth. The organism could not be isolated from infected leaves or petioles 1 week after treatment, but was found to be still active in some infected stolons. On March 30, 1953, green weight yields on 8 paired blocks of treated and non-treated areas averaged 13,886 lb. per acre on the treated compared to 4,432 lb. on the non-treated. In 1954 dosage and spray timing trials indicated that applications of 12–15 lb. per acre of active material should be applied just after the peak of apothecial production. Sprays prior to this time did not suppress the formation of apothecia.

Resistance to the dwarf disease in alfalfa. HOUSTON, BYRON R., AND ERNEST H. STANFORD. The dwarf disease of alfalfa (Pierce's disease virus) is an important factor in rendering alfalfa fields unproductive in about ⅓ the acreage in California. Sixteen varieties of alfalfa (*Medicago sativa*) tested under field conditions showed no difference in relative degree of susceptibility. Eighteen alfalfa varieties, *M. falcata,* and *M. glutinosa* inoculated in a screened cage infested with viruliferous leafhoppers (*Draeculacephala minerva*) showed 100 per cent susceptibility. About 800 plants of California Common alfalfa showing tolerance to the disease in field plantings were selected, transplanted in isolation and allowed to intercross. Seed from each of 343 plants was planted in 3 field plots in replicated 3-row blocks. At the end of the third growing season the average survival of California Common was 8.0 per cent, whereas some selections showed normal growth with 70–80 per cent survival. Cuttings from 320 of the original selections were inoculated to test their resistance. Several of these clonal lines were found to be highly resistant. Selfing with subsequent loss of vigor renders progeny more susceptible than progeny from inter-crosses involving the same parents. Thirty-one parent plants were selected and used as the base for dwarf-resistant California Common 49.

Vulnerability of Rhizoctonia solani in relation to turf brown patch control. HOWARD, F. L., AND BARBARA CHAMPLIN. Inconsistent results with fungicides for control of brown patch of bentgrasses stimulated a study of the toxicity of chemicals and biological activity of antagonistic fungi against mycelium and sclerotia of *R. solani.* Comparison of similar concentrations of mercury chlorides, thiram, and a cadmium-chromium mixture by 4 laboratory techniques yielded contradictory results. A method was evolved in which sclerotia or mycelia growing on agar are exposed to a time-dosage series of the candidate fungicide and then transferred aseptically to water-agar for incubation. Mycelia were killed only by mercury chlorides, sclerotia only by cadmium-chromium mixture; thiram was ineffective. Induced germination of sclerotia by wetting prior to fungicide application resulted in attrition. Two or 3 treatments at 4–7 day intervals reduce the sclerotial inoculum by chemotoxic action and by permitting cellulose-decomposing fungi to feed on the softened sclerotia. Cadmium-chromium mixture is apparently selectively fungitoxic to *Rhizoctonia* but not to its antagonists. Whereas mercury chlorides are specific for *R. solani* hyphae attacking bentgrass foliage, watering the turf prior to application of certain fungicides is required for effective prevention of brown patch.

The effect of aphid toxins on Cymbidium orchid flowers. JENSEN, D. D. Aphids feed reluctantly on Cymbidium foliage but natural infestations frequently occur on floral parts.

The lily aphid (*Myzus circumflexus*) usually selects the younger buds and feeds primarily on the under side causing the ventral sepal particularly to pull away from the rest of the bud prematurely. It then becomes distorted by twisting and by rolling inward of the lateral margins. Such sepals remain dwarfed and distorted, after the rest of the flower has opened normally, turn yellow and die prematurely. Pale circular spots develop at the sites of feeding. In the vicinity of its feeding site the foxglove aphid (*M. solani*) causes areas in the sepal to turn yellow with a rose-colored boundary. Severely affected buds later become discolored throughout and drop prematurely. *M. ornatus* and the green peach aphid (*M. persicae*) cause small chlorotic spots which may persist after the flowers open. Subsequently some of the spots may develop a water-soaked appearance. Large numbers of *M. ornatus* can also cause some premature pulling away of the sepals. Feeding on sepals alone does not cause specific petal symptoms.

Leafhopper transmission of the Napa strain of cherry buckskin virus from cherry to peach. JENSEN, D. D., AND H. EARL THOMAS. The Napa (California) strain of cherry buckskin virus was transmitted from infected Royal Ann cherry on Mazzard root to 42 of 181 Lovell peach seedlings by the leafhopper *Colladonus geminatus* (*Van Duzee*). After feeding for 37 days on the diseased tree, the insects, tested in groups of 20, were transferred weekly to healthy trees until all insects had died July to September 1953. None of the previously healthy trees fed on during the first 2 weeks developed the disease. The first transmissions occurred 51–57 days after the insects were first caged on the inoculum tree. Symptoms developed in 33 test trees in the fall of 1953 and in 9 additional trees in the spring of 1954. Although some trees ultimately developed symptoms indistinguishable from those caused by yellow leaf roll virus, the Napa virus was generally milder in its effects and was slower to produce both current season symptoms and second year symptoms. Moreover, *Colladonus geminatus* acquires and transmits the Napa virus in a much lower percentage of cases than when the same number of leafhoppers are tested with yellow leaf roll virus.

Physiologic races of the leaf rust of wheat, Puccinia rubigo-vera tritici, in Mexico. JOHNSTON, C. O., H. R. HEWLETT, AND N. E. BORLAUG. Studies of 153 leaf rust collections made in Mexico in 7 years of the period 1944–1953, inclusive, revealed the presence of 30 physiologic races. Several races were very similar to other well-known races and therefore were grouped together under the number of the latter. This reduced the number to 11 important and distinctly different race-groups. Physiologic race 11 proved to be by far the most important one, comprising 51.2 per cent of the total of 457 isolates. It was the most abundant race in each of the 7 years and in all but 1 of the 8 states from which collections were received. This race is abundant in the Pacific Coast states but relatively unimportant in other wheat-growing areas of the United States. A small increase in the prevalence of race 11 in central United States in recent years indicates the movement of some leaf-rust spores from Mexico into the United States. On the other hand, a sharp increase in recent years in the prevalence of race 5 in Mexico strongly suggests the movement of leaf rust spores from the United States into Mexico.

Downy mildew of orchard grass caused by Sclerophthora sp. JONES, WALTER. In June 1948, an unreported downy mildew disease of orchard grass (*Dactylis glomerata* L.) caused by *Sclerophthora* sp. was found in the test rows at Saanichton, near Victoria, British Columbia. On February 22, 1954, when the minimum and maximum temperatures were 39.5° and 46° F. respectively, numerous sporangia of the fungus were present on diseased leaves. On leaf blades, symptoms appeared as light-brown, oval-elongate areas and interveinal streaks. A reliable symptom was the presence of whitish 'clumps' of sporangia around the stomata on the ventral surfaces of the leaves. Only occasionally were the inflorescences affected. Sporangiophores, short, emerging through stomata, simple, with sporangia produced in a close monochasial sympodium. Sporangia numerous, hyaline, papillate, obpyriform, with pedicels persistent. Length 30.5–38μ, width 15–19μ. Oogonia within mesophyll cells, numerous, subglobose to spherical, diameter 34–45μ, wall 1.9–3.8μ, sinuous, golden to amber-brown. Antheridia paragynous. Oospores spherical, diameter 34μ, wall 1.5–3μ, confluent, with oogonial wall. The optimum temperature for zoospore emergence was approximately 10° C. A few discharged at 1° C. but none above 20° C. The morphological characters of the fungus and its low-temperature growth habit indicate that it is a new and undescribed species of the recently described genus *Sclerophthora.*

Further studies on the phosphate effect in plant virus inoculations. KAHN, ROBERT P., AND N. D. SCHACHTNER. The phosphate effect, i.e., increased infection due to the presence of 0.1 M potassium phosphate buffer at pH 7 was investigated at 2 phases in the infection process of tobacco-mosaic and southern bean-mosaic viruses on detached Pinto bean leaves. When the inocula did not contain added phosphate buffer, abrasion with buffer produced more infection than either dry- or water-abrasion. A dip in buffer, or no dip, after dry abrasion but preceding inoculum application resulted in more infection than a water-dip. Thus, the phosphate effect was evident both at time of abrasion and immediately after abrasion. When the inocula contained added phosphate, abrasion with buffer resulted in about the same amount of infection as either dry- or water-abrasion. A difference in response due to presence or absence of phosphate was not anticipated since it was present in the inoculum for all treatments. A dip in either phosphate buffer or in water, following dry abrasion but preceding inoculation, resulted in equal infection but both were considerably less than the no-dip control. Thus, the phosphate effect was not apparent.

Differential longevity in spores of physiologic races of Tilletia caries. KENDRICK, E. L. The viability of spores of 11 races of *Tilletia caries* stored as smut balls and as powdered spores in the laboratory for 18 years was compared with the viability of spores from current stocks of the same races stored as smut balls. In all races, the germination rate was slower in old spores than in spores of current stocks. Furthermore, this reduced rate of germination was greater in some races than in others. For example, spores from current stocks of T-6 germinated about 50 per cent in 4 days whereas a comparable percentage of germination in the old spores of this race required 15 days. In contrast, maximum percentages of germination in T-8 required 6 and 9 days for the fresh and old spores, respectively. Complete loss of viability was indicated for spores of T-11 stored in ball form. Fresh spores of this race germinated above 50 per cent in 8 days whereas old spores from bunt balls failed to germinate. However, the old powdered spores of T-11 germinated as much as 7 per cent in 3 weeks. This suggests that form of storage may influence longevity but the results in general were inconsistent with respect to this factor.

Chemical protection of plants from ozonated olefin (smog) injury. KENDRICK, J. B., JR., JOHN T. MIDDLETON, AND ELLIS F. DARLEY. Plant injury characteristic of that found in the San Francisco Bay area and southern California caused by exposure to ozonated gasoline or hexene-1 can be prevented by sprays or dusts of zineb, ferbam, ziram, maneb, or thiram on the plant surfaces. Bordeaux at 3-3-100, lime, talc, and pyrophylite applied to the test plant, pinto bean, *Phaseolus vulgaris* L., were not effective in preventing this injury. Bean plants sprayed with dichlone or chloranil were damaged more than the nontreated controls upon exposure to the phytotoxicant. Zineb dusts of less than 10 per cent active and sprays of less than 2 gm. active per liter were not so effective in preventing injury as more concentrated forms when exposed to approximately 0.4 p.p.m. concentration of the phytotoxicant. Plants dusted with zineb or thiram were increasingly injured with increase in time between dusting and exposure to the phyto-

toxicant, to a maximum of 16 days. Likewise, the injury increased progressively on the protected plants with increased toxicant-exposure times. The protection phenomenon was evident only when the lower leaf surface was adequately covered by effective chemicals.

Physiologic specialization in Aphanomyces euteiches. KING, THOMAS H., AND HOWARD BISSONETTE. Six isolates of *Aphanomyces euteiches* were obtained from diseased peas grown in infested soil collected from different parts of Minnesota. These isolates were single spored and grown at 5 different temperatures in flasks containing a sterile decoction of maize kernels in tap water. The single-zoospore cultures differed in time required for sporulation and in ability to produce zoospores and oospores. The isolates also differed in ability to attack different varieties and selections of canning peas. Two isolates were highly pothogenic, 2 were moderately so, and 2 were non-pathogenic on a variety commonly grown in Minnesota. In addition, the highly pathogenic isolates were virulent on a selection previously considered tolerant. One weakly virulent isolate caused seed deterioration and seedling damping-off but did not attack mature plants of the varieties tested.

A unique greenhouse facility for plant disease research. KINGSOLVER, CHARLES HUSTON, AND JOHN EDWARDS MITCHELL. The greenhouse consists of four 25 × 60 ft. houses and six 10 × 20 ft. cubicles with exterior walls and roof of thermopane, and interior partitions of plate glass. Each unit is individually air conditioned and a program type recorder-controller maintains temperature and humidity in a pre-set pattern. Temperatures were maintained at 75° during the summer without the application of white wash, when outside temperature was near 100°F. Maximum air velocity at plant level approximates 1 mile per hour. Incoming and exhaust air are filtered through electro-matic filters and deep bed filters respectively. Cross contamination between the various units of the building is minimized by maintaining the air pressure in all working spaces negative to that of the central passageway. This pressure differential, coupled with special personnel change-room facilities and material and equipment handling procedures, makes possible the elimination of contamination to or from the outside. The building contains a room with a settling tower and dew chambers for inoculation studies, and laboratories fitted for cultural, histological, and physiological studies as well as the usual planting and soil handling facilities.

Relation of rainfall to occurrence of apple scab and sooty blotch. KIRBY, R. S. Counts of disease occurrence in Pennsylvania during the past 25 years were made of 3,075,951 apples in 8,612 orchards. The amount of scab occurring in any year in unsprayed orchards was proportional to the amount of rainfall in April and May and to a lesser extent in June and July. When the 25 years was divided into periods of the first 10 years, the second 10 years and the last 5 years, the average amount of scab in unsprayed orchards in any period varied by less than 1 per cent from that in any other period. The amount of sooty blotch occurring in any year in unsprayed orchards was proportional to the amount of rainfall occurring during July, and to a lesser extent in August and September. When the 25 years was divided into periods of the first 10 years, the second 10 years and the last 5 years, the average amount of sooty blotch in any period varied but 2 per cent from that in any other period.

Comparative seed damage within paired isogenic lines of flax. KOMMEDAHL, T., AND J. O. CULBERTSON. Fourteen pairs of isogenic lines of flax were obtained by selection from crosses of yellow- by brown-seeded varieties. Plants heterozygous for seed-coat color were selected in each segregating generation up to and including F_{10}, when pure yellow- and brown-seeded lines were increased for yield trial. Yellow-seeded lines contained 6–15 times more split seed coats, up to 2.5 times more cracked seed, and up to 3.3 times more blighted seed than the corresponding brown-seeded lines. Total seed damage was 2–5 times greater in the yellow-seeded lines. On 2 culture media, Alternaria was isolated more frequently from brown- than from the yellow-seeded line of a pair. Certain other fungi predominated on yellow-seeded lines. Germination of brown-seeded flax on blotters was about 10 per cent higher than that of the yellow. Deformed seedlings were more common in the yellow-seeded lines of each pair. Stands of untreated seed in soil were 1.3–2.6 times higher in the brown- than in the corresponding yellow-seeded line. Response to seed treatment (captan) was greater in the yellow- than in the corresponding brown-seeded line; yellow-seeded lines increased 30–160 per cent in stand with treatment compared to 1–60 per cent increase for brown-seeded lines.

Importance of temperature control in studies of uredial infection with Puccinia graminis tritici. LANGE, C. T., AND C. H. KINGSOLVER. Air temperatures of 9 controlled dew chambers were adjusted to approximately 60°, 65°, 70°, and from 70°–85°F. at 2½° intervals. Pots of 7–10 day old Baart wheat seedlings trimmed to primary leaves were placed, leaves horizontal, in the incubation chambers. Twenty mg. of uredospores were discharged upwards into each closed chamber by an instantaneous blast of compressed gas from a CO_2 type target pistol fitted with a special discharge tube. Thus uniform deposition of controlled amounts of uredospores on horizontally oriented seedling leaves was obtained. By manipulation of chamber controls, free water was deposited and held on leaves of test plants for 20 hours. Pustule counts were made after holding plants 10–14 days in a greenhouse at 75°F. Analysis of variance of all tests yielded highly significant differences in pustule counts between temperatures of any 1 test. Very little infection occurred at 85°F. or at 60°F. Maximum pustule counts in 4 tests with races 11, 15B, 17, 38, 49, 56, and 139 occurred at an average temperature of 73.5°F. Infection at incubation temperatures below the optimum decreased about 10 per cent for every degree Fahrenheit drop. Slightly larger percentage decreases in pustule numbers for every degree above the optimum were recorded.

Comparison of isolates of Phytophthora infestans at different temperatures. LARANCE, ROBERT S., AND W. J. MARTIN. Experiments were designed to compare the growth of 13 isolates of *Phytophthora infestans* (Mont.) de Bary under uniform conditions of media and inoculum at 3 different temperatures (21° C., 23° C., 27.5° C.). Highly significant differences were recorded among isolates in the diameter of colonies at each temperature. All isolates grew better at 23° C. than at the other temperatures. Considerable decrease in growth was observed at 27.5° C. Another series of experiments was made to determine survival of 13 isolates when exposed for varying periods of time at 32.5° C. and 35° C. Each isolate was exposed to each temperature for 16, 32, and 48 hours. All isolates survived the 3 exposures at 32.5° C. At 35° C. only 12 of the isolates survived the 16-hour exposure, 8 survived the 32-hour and 5 survived the 48-hour exposure. All isolates that survived the 48-hour exposure at 35° C. were isolates obtained from the southern part of the United States.

Physiologic races of Piricularia oryzae. LATTERELL, FRANCES M., E. C. TULLIS, R. J. OTTEN, AND A. GUBERNICK. Among 7 isolates of *Piricularia oryzae* Cav. from 3 rice-growing areas of the United States, 2 physiologic races were distinguished on the basis of their differential pathogenicity to certain varieties of rice. The races could be separated on the host reaction of the varieties Zenith and Caloro. Zenith was severely attacked by the isolates from Florida, but was immune from the isolates from Arkansas and Louisiana. Caloro was very susceptible to the Arkansas and Louisiana isolates, but was immune from those from Florida. Differential reactions between the 2 races were observed on 4 other varieties of rice. Five distinct lesion types were charactrized on the varieties tested. Two varieties, LaCrosse and C.I. No. 8970, were found to be impure by inoculation with the Florida strain. Approximately 1 plant in 5 of LaCrosse was highly susceptible to this strain, while the majority of plants were immune. Approximately 1

plant in 3 of the variety C.I. No. 8970 was immune, while ⅔ of the plants were susceptible. In the latter variety, immune plants had purple auricles, whereas susceptible plants had colorless auricles. In this case, the plants of each pigmentation type bred true, indicating varietal impurity rather than segregation.

The nature and function of the exudate produced by Xanthomonas phaseoli. LEACH, J. G., VIRGIL GREENE LILLY, AND H. A. WILSON. Exudate comparable to that produced in nature is formed abundantly on 2.5 per cent glucose agar. Aeration is essential for maximum production in liquid medium, from which the exudate is precipitated by acetone. Exudate produced on agar has water content of approximately 96 per cent, about 3.5 per cent of which is bound water. The exudate is a polysaccharide-like substance with a molecular weight above 19,000,000, as determined by light-scattering measurements. It has high anionic mobility and contains sugar residues. It loses viscosity on autoclaving and by acid hydrolysis. Maximum live-cell content is reached on the fourth or fifth day on glucose agar, the stationary phase lasting until the seventh day, after which the count decreases rapidly. Approximately 50 per cent of the cells survived in drops of exudate collected before the seventh day and dried on coverglasses over Drierite. Twelve per cent were still viable after storage for 440 days at 5°C. and 20 per cent relative humidity. The exudate was toxic to excised seedlings at a concentration of .5 per cent but not at 0.5 per cent. Hydrolysis to reduce the size of the molecules did not affect the toxicity.

Yields and off-flavor of potatoes and carrots grown on plots receiving annual soil treatments. LEAR, BERT, W. F. MAI, M. B. HARRISON, AND H. S. CUNNINGHAM. Seventeen chemicals comprising 40 treatments were applied to replicated plots, beginning in the fall of 1949. Chemicals included: D-D and Dowfume N (dichloropropane-dichloropropene mixtures), ethylene dibromide, chloropicrin, dichlorobutenes, Arasan (thiram), Dithane Z-78 (zineb), Dow 9B (zinc trichlorophenate 32 per cent), carbon tetrachloride, calomel, xylene, stanisol, Vancide 30W, propylene dichloride, Eston thinner, and Westvaco thinner. A 10-year dosage for each material was applied once in the fall of 1949 and annual rates applied in the fall of 1949, 1950, and 1951. Yields of potatoes (Katahdin) and carrots (Nautis) were determined yearly starting in 1950 and random samples of each removed from each plot for off-flavor determinations. Replicate samples from each treatment and check, with a known check for comparison, were cooked and submitted by number to a taste panel. The only annual dosage resulting in yield reduction was 100 lb. of Dow 9B. The reduction occurred in 1950 and 1951 but not 1952. Both crops were significantly reduced on plots receiving 1 application of 500 lb. of D-D, Dowfume N, and dichlorobutenes and 1000 lb. of Dow 9B. This reduction persisted 2 years for Dow 9B and 1 year for the others. No potato plants emerged on the dichlorobutene treated plots. Off-flavor was detected only in the 5000 lb. dosage of D-D and Dowfume N after 1 year but not after 2 years.

Irradiation of Venturia inaequalis for the production of mutants resistant to fungicides. LEBEN, CURT, D. M. BOONE, AND G. W. KEITT. Conidia of wild-type line 365-4 were irradiated with ultraviolet light. Approximately 290,000 spores, of which 27 per cent survived the treatment, were plated on an agar medium containing antimycin A-35, helixin B, thiram, or sulfur at 1.25, 25, 2.5 and 20 μg/ml., respectively, amounts 20 times those required for an inhibition point of 365-4. No mutant resistant to helixin B, thiram, or sulfur was found. One colony grew on the antimycin medium. This line, designated 3164, was retested 3 times on antimycin media. Growth was not inhibited even at 320 μg of antimycin per ml., some 5,000 times the amount required for the inhibition point of 365-4. 3164, which grew more slowly than 365-4 on a control medium containing no antimycin, was mated with a wild-type line. When the 8 ascospores from each of 44 asci were cultured on the control medium, 4 produced colonies like the wild-type parent and 4 produced slow-growing colonies like 3164 (43 per cent second division segregation). In all of 10 asci tested, the slow-growing lines were resistant to antimycin, whereas the wild-type lines were inhibited by the antibiotic. Pathogenicity tests of 3164 and its progeny are in progress.

A genetic basis for rust reaction on corn. LEROUX, P. M., J. G. DICKSON, A. L. HOOKER, AND G. F. SPRAGUE. Cooperative investigations on pathogenicity in *Puccinia sorghi* and related species, and resistance in *Zea mays* and other hosts were initiated in 1952. *Oxalis* species were used to determine genes for pathogenicity on the aecial hosts and as a means of inbreeding and outbreeding the pathogen. The 2 classes of disease reaction used were resistant (chlorotic to necrotic flecks—0; to 1—) and susceptible (fully developed, sporulating pustules—2 to 4). Sixty-eight urediospore and 13 aeciospore isolates originating from various locations in North America were tested on corn seedlings of lines from North and South America. Investigations to date suggest 8 or more gene pairs in corn conditioning rust resistance to at least 15 gene groups of the pathogen. Only 1 line of corn (from Peru) was immune from all isolates of the pathogen. At least 2 gene groups for pathogenicity on the *Oxalis* hosts have been isolated. A study of the genes for pathogenicity on both the uredial and aecial hosts suggests that *P. purpurea* and *P. polysora* possess genes in common with *P. sorghi*. The genes conditioning rust resistance are being established as isogenic lines in several corn inbreds to differentiate fungus genotypes and study nature of rust resistance.

Pathogenicity tests with the clover-root nematode. LINFORD, M. B. Use of steam-sterilized soil increased the apparent pathogencity of *Heterodera schachtii.* var. *trifolii* to white clover in a greenhouse experiment with soil initially free from *Heterdera.* Seven replications in 6-in. pots compared non-sterile vs. steam-sterilized soil, with and without infestation with 25 washed, non-desiccated cysts per pot. Fresh weights of tops from successive harvests during 14 months averaged slightly but not significantly greater in non-sterile than in sterilized-soil checks, despite an active population of *Paratylenchus* sp. in the non-sterile soil. In the 2 infested series, early development of chlorosis and stunting were similar, but maximum severity was greater in soil sterilized before infestation. Weights of 10 weeks' growth harvested 46 weeks after planting in the infested non-sterile soil averaged 28.4 per cent of the checks; in the infested sterilized soil, only 6.2 per cent of the checks. The lesser severity in the non-sterile soil appears to result from partial biological control of nematodes by organisms initially present in that soil but destroyed by sterilization. Tested in sterilized soil, Ladino clover proved highly susceptible, but red clover growth was not reduced significantly during 12 months despite multiplication of the nematodes.

Vector feeding relative to leafroll resistance in potato varieties. LOCKE, S. B. Aphid counts on potato varieties conducted during 5 seasons at Pullman, Washington, revealed no correlation betweeen the vector population occurring on a variety and the amount of leafroll infection taking place in that variety. Cage experiments, wherein uniform numbers of aphids were confined to small leaf areas on 3 leafroll resistant and 2 leafroll susceptible potato varieties, revealed no significant differences with respect to survival or increase of the aphids on these varieties. However, highly significant differences occurred in amount of injury to the leaf tissues resulting from aphid feeding. The 2 susceptible varieties showed low aphid-feeding-injury indexes, and 2 of the resistant varieties showed high aphid-feeding-injury indexes, suggesting that at least 1 type of field resistance is associated with sensitivity to aphid feeding. A third leafroll resistant variety showed a low aphid-feeding-injury index, indicating that a second type of field resistance is operating.

Effect of temperature on the development of ring rot. LOGSDON, CHARLES E., AND C. J. EIDE. Temperature was a major factor contributing to the ring rot (*Corynebacterium*

sepedonicum) epiphytotic in Alaska in 1953. The inoculum potential built up during previous cool seasons plus the above normal temperatures of 1953 brought about losses of from 10 to 15 per cent in the potato crop. Optimum temperature for growth of both an Alaska isolate and a Minnesota isolate of the bacterium in culture was about 15° C. on agar and also in liquid culture. Optimum soil temperature for the development of the disease (using the Minnesota isolate) was approximately 25° C. At 16° C. 97 per cent of the plants became infected, only 81 per cent developed symptoms and 28 per cent completely wilted. At 25° C. 100 per cent of the plants became infected and 87 per cent wilted completely. There was little difference in the percentages of infected tubers at these temperatures. Fifty per cent were infected at 16° C. and 55 per cent were infected at 25° C. soil temperature.

The relation of the tobacco cyst nematode to tobacco growth. LOWNSBERY, B. F., AND B. G. PETERS. In an outdoor pot experiment height and final weight of Connecticut 15 shade tobacco plants were inversely proportional to the logarithm of the initial density of viable encysted *H. eterodera tabacum* larvae in soil in which the plants were grown. This was true over the range 50–3200 larvae per gm. of soil. Every 10-fold increase in density led to a loss in plant weight of 139 gm. for normally fertilized plants, and 25 gm. for smaller, unfertilized plants. The highest density which maintained itself was 1000 larvae per gm. of soil. At this density reduction in weight of normally fertilized plants was 25 per cent. In a field plot experiment, Connecticut 15 and Connecticut 49 shade tobacco plants grown 2 months in dichloropropene-dichloropropane treated plots (0.1 viable encysted *H. tabacum* larvae per gm. of soil) were 15 and 22 per cent taller, respectively, than controls grown in untreated plots (103 viable encysted *H. tabacum* larvae per gm. of soil). In this experiment, nematode reduction was confounded with other fumigant effects, e.g. ammonia nitrogen was raised and nitrate nitrogen lowered. During the 1953 season the *H. tabacum* population in untreated plots rose to 359 viable encysted larvae per gm. of soil.

The effect of root-knot nematodes on the expression of granville wilt resistance in tobacco. LUCAS, G. B., J. N. SASSER, AND A. KELMAN. The relationship between root-knot nematodes and severity of Granville wilt in wilt-resistant tobacco was investigated in greenhouse experiments. Tobacco seedlings of a moderately resistant variety, Dixie Bright 101, growing in pots of steamed soil were exposed to each pathogen alone and in combination by the following treatments: 1) 50 ml. of a suspension of *Pseudomonas solanacearum* poured around the roots, 2) approximately 100 gm. of soil infested with eggs and larvae of *Meloidogyne incognita* var. *acrita* placed around the roots, and 3) both *P. solanacearum* and *M. incognita var. acrita* added to the soil. Seven days later the percentage of plants showing wilt symptoms in the respective treatments was as follows: 1) 5, 2) 0 and 3) 90. After 22 days, only 10 per cent of the plants in treatment 1) were dead, whereas all plants in 3) were dead. No plants in treatment 2) had any above ground disease symptoms. In 3 other similar tests bacterial-wilt resistant plants growing in soil infested with both pathogens invariably exhibited wilt symptoms earlier than plants growing in soil infested with the wilt pathogen alone. These results indicate that Granville wilt severity is increased in Granville-wilt-resistant tobacco growing in soil infested with both *M. incognita* var. *acrita* and *P. solanacearum*.

Studies on the mechanism of fungicidal action of the ethylenebisdithiocarbamates. 1. Practical considerations. LUDWIG, R. A., AND G. D. THORN. Reactions involved in formation of the metallic ethylenebisdithiocarbamates were found to be more than simple precipitations. Water suspensions of manganese ethylenebisdithiocarbamate, like the parent nabam, readily yielded on aeration a product containing the highly fungicidal compound ethylene thiuram monosulphide (E.T.M.) The pure zinc ethylenebisdithiocarbamates used proved to be relatively non-fungicidal when suspended in distilled water and could not be converted to E.T.M. Addition of a small amount of alkali, however, improved solubility, increased fungicidal activity, and permitted conversion to E.T.M. The iron-nabam reaction was found to be more complicated but probably also yielded E.T.M. Results obtained by standard analytical methods are not necessarily related to fungicidal activity.

Changes in the viable golden nematode population of the soil while growing potatoes every year. MAI, W. F. The soil population of viable golden nematodes (*Heterodera rostochiensis* Wr.) was measured before and after cropping with potatoes in 6 experimental areas on Long Island, New York. Data were taken during 1952 and 1953; 2 warm, dry growing seasons. Soil type varied from a sandy loam to a silt loam. Numbers of cysts containing living larvae varied from 6 to 80 per 2 oz. of air-dried soil, in soil samples taken before cropping. Changes in the numbers of cysts containing viable larvae as a result of growing a crop of potatoes were expressed at ratios. The ratios varied from 0.13× to 7.2×. A decrease in living larvae was obtained 9 times and an increase was recorded 3 times. In 3 of the experimental fields, numbers of cysts with viable larvae decreased during each of the 2 consecutive growing seasons. In a given experimental area, the percentage decrease was directly proportional to the number of cysts with viable larvae in the soil before cropping.

The biological activity of alternaric acid. MAITLEN, GENE, AND GEORGE A. GRIES. Tomato cuttings placed in aqueous solutions of alternaric acid (AA) develop leaf and stem lesions followed later by general wilting of the shoot. Prior to expression of these macroscopic symptoms, there is an increase in CO_2 production. An increased oxygen consumption is obtained with excised tomato leaves in the Warburg apparatus, but no shift in the respiratory quotient occurs. AA disturbs the water balance of tomato cuttings as evidenced by an increase in transpiration and water-uptake. Respiration and transpiration rates increase concomitantly and typical leaf symptoms occur 2 hours after their initial rise. Wilting is not apparent until the respiration and transpiration rates start to decline. The water absorption rate of the treated cuttings does not increase until just prior to or concomitant with wilting. A 0.25 saturated solution of AA in deionized water (approx. 10 p.p.m.) increases permeability and decreases respiration of beet root slices with no change in the respiratory quotient.

Comparative effect of fungicides on oxygen uptake and germination of spores. MCCALLAN, S. E. A., LAWRENCE P. MILLER, AND RICHARD M. WEED. The effect of 20 fungicides on exogenous oxygen uptake and germination was determined for spores of *Neurospora sitophila, Monilinia fructicola, Myrothecium verrucaria, Alternaria oleracea, Aspergillus niger, Rhizopus nigricans, Cephalosporium acremonium* and *Stemphylium sarcinaeforme.* After exposure of spores to soluble fungicides in a microrespirometer for 5 hours, samples were taken for germination. In general, oxygen uptake was decreased except for *N. sitophila* for which an increase occurred with silver, copper, and phenol. Germination was a more sensitive test for toxicity than oxygen uptake as shown by steeper dosage-response curves and lower ED50 values expressed as μg. of toxicant per mg. of spores. Effect of water insoluble fungicides was determined by pretreatment of spores for 15 minutes in dilute acetone solution (not over 2 per cent). Chloranil, dichlone, captan, glyodin, and ferbam increased oxygen uptake of *N. sitophila* at low concentrations and decreased it at higher concentrations. With *M. fructicola* there was no increase of oxygen uptake and ED50 values for germination were higher than for oxygen uptake. Nabam in pretreatments was without effect on oxygen uptake or germination. An excess of wettable sulfur in the respirometer increased oxygen uptake of all species except *S. sarcinaeforme* and had no effect on germination except for *M. fructicola.*

A virus disease of corn present in Lilium speciosum from Japan. MCWHORTER, F. P., AND H. H. MILLSAP. *Lilium speciosum* var. *rubrum,* imported in 1952 from Japan, de-

veloped conspicuous white cell flecking besides the usual lily virus mottles. A virus transferred from flecked leaves to *Zea mays* var. Golden Cross Bantam caused fleck symptoms only when returned to *L. speciosum* seedlings. The virus has a very narrow host range. Transfer was not obtained to burley tobacco, cucumber, oats, barley, wheat, rice, and 3 varieties of sorghum under conditions where control transfers from corn to corn averaged more than 85 per cent. The virus is unstable, loses activity rapidly in expressed juice after a 20-minute interval at 21°C., and is non-infectious at 1/100 dilution. These data indicate this virus to be neither one of those described in corn, nor a strain of cucumber mosaic virus. However, it transfers readily from corn to *L. longiflorum* var. Croft and produces severe fleck disease symptoms in the lily. In corn, the symptoms are not flecking, but severe blanching and chlorotic streaking. It is concluded that the virus is an undescribed species related to cucumber mosaic virus and worthy of serious consideration by those who grow imported speciosum lilies in areas where corn is a principal crop.

Histochemical studies on differences in rate of germination of common and dwarf bunt spores. MEINERS, JACK P. Spore germination in dwarf bunt from wheat and grasses requires longer incubation at a lower temperature than germination in common bunt of wheat. Studies were undertaken to determine whether this differential viability is due to differences in the chemical nature of the spore wall. This is a report on a histochemical analysis for chitin using the chitosan reaction test. Chitin was found in both common and dwarf bunt types (*Tilletia caries* and *T. foetida*) indicating, therefore, that the differential viability of common and dwarf bunt spores is not related to differences in chitin content. It was also evident from this study that the spores have a double wall. The chitin is confined to the inner wall, which stained purple, while the outer wall remained colorless, reflecting the absence of chitin. In addition to differing chemically, the physical structure of the inner and outer walls was found to be distinct. The inner wall, whose outer surface is smooth in both species, can be separated from the outer wall by crushing the spores.

Response of cowpea varieties to strains of alfalfa mosaic virus. MILBRATH, J. A., AND F. P. MCWHORTER. When 9 varieties of cowpea were inoculated with 26 strains of alfalfa mosaic virus, local lesions developed within 3–8 days after inoculation. Sixteen strains of virus produced either sharply defined and small or large black, irregular and immarginate local lesions. The other 10 strains showed indefinite local lesions of variable necrotic and chlorotic spots, often in the form of well-defined rings. The first group failed to become generally systemic, although some strains developed a necrosis which spread through the veins into the petiole and main stem. The 10 strains with indefinite local lesions all became systemic and caused variable degrees of stunting, wilting and dying, and a general chlorotic mottling of the trifoliate leaves. In some cowpea varieties symptoms were more severe than in others, but in general each strain formed a definite pattern on all varieties tested. Some strains produced brilliant golden mottles and others a pale green chlorotic pattern. Genetic virus-like abnormalities confused symptom interpretation especially in some varieties, but the varieties Black Cowpea and Virginia Black Eye gave reliable results and are suggested for indicator varieties. The cowpea appears to be a valuable host for identifying alfalfa mosaic and for placing the many strains into distinct groups.

Quantitative differences in the nucleic acid composition of cherry leaves collected from ring-spot-infected and disease-free Montmorency cherry. MILLIKAN, D. F. A preliminary study of the nucleic acid content of Montmorency cherry indicates that the nucleoprotein associated with ring spot virus complex is of the ribose type. Leaves of the Mont Stark strain of sour cherry were collected and lyophilized. Nucleic acids were extracted from cherry powder with a modification of the perchloric acid method described by Ogur and Rosen. To remove impurities that interfered with the ultraviolet absorption peak for nucleic acids, it was necessary to extract the cherry powder first with acetone in a Waring blendor followed by 3 extractions with 70 per cent ethyl alcohol. Leaves from trees infected with Goldman 20-5, S-5009, S-16, and Barnard 1-12 strains of the ring spot virus complex contained approximately 50 per cent more RNA than the disease-free control. RNA/DNA ratios also showed approximately 50 per cent greater values for the infected material.

Bark splitting, a possible virus disease in apple. MILLIKAN, D. F., AND H. W. GUENGERICH. For several years a disorder has been observed on double worked Virginia Crab characterized by severe longitudinal cracking on the stem between the ground line and the first scaffold branches. This may be accompanied by a long willowy growth of the top-worked variety. First symptoms appear as 1–3 vertical cracks in the bark about 3–4 in. long. Eventually, the width of this splitting may be as much as 2–3 in. tapering at both ends. During the past greenhouse season, buds from 3 questionable sources and 3 sources considered as healthy were chip-budded into apple seedlings, Virginia Crab, *Prunus tomentosa*, *P. besseyi*, quince, Marianna, plum, pear seedlings, peach seedlings and Montmorency cherry. Two of the 4 tomentosa plants budded with an infected Virginia Crab bud showed a striking mottling of the foliage. This mottling differed from that of the ring spot on this host in that the affected areas did not become necrotic and fall out. Phloroglucinol tests for the rubbery wood described by Beakbane and Thompson on dormant wood were positive. However, the disorder differed from the description of rubbery wood and the exact relationship, if any, is yet to be established.

A variable temperature incubation chamber permitting controlled deposition of dew. MITCHELL, J. E., AND EDWARD CHERRY. A "dew chamber" has been developed to provide a wide range of incubation temperatures and densities of dew deposition. The incubation conditions are controlled by regulating the wall temperature and the temperature of a tray of water in the bottom of the cabinet. The former is achieved by circulating tempered water through serpentine type evaporator plates attached to the interior walls of the cabinet. The temperature of the water in the evaporator plates is regulated by a thermostatically controlled blending valve within the limits of a hot and cold water supply. The water pan temperature is controlled by an immersion heater in conjunction with a Bristol thermometer-controller. The plants are placed on a rack a few inches above the water. The density and rate of dew deposition is a function of the temperature differential between the walls and the water surface; the greater the differential, the more rapid and heavier the deposit. The air temperature is a resultant of the 2 temperatures and approximates the mean between them. The dew chamber has been used successfully at temperatures from 55° to 95°F. and is independent of season. It is simple to operate and maintain.

Temperature requirements for identification and differentiation of races 49 and 139 of Puccinia graminis tritici. MOHAMED, HOSNI A. Races 49 and 139 of *Puccinia graminis tritici* Erikss. & Henn. are virulent on some of the Kenya wheats and their derivatives used in wheat breeding programs. During prolonged periods of hot weather in summer there were difficulties in differentiating between these 2 races on the 12 differential varieties of wheat. From comparative studies of many isolates of each race at controlled temperatures, it was found that race 49 produces its characteristic infection type 4 on Marquis and Kota wheats at all temperatures from 60 to 90° F., whereas race 139 produces its characteristic infection type 2 on Marquis and Kota only at temperatures between 60 and 72° F. Almost all isolates of race 139 produce infection types 3 and 4 on these 2 varieties of wheat at 85 to 90° F. and thus look like race 49 at high temperatures. The reaction of the 2 wheats to race 139, therefore, changes from resistant to susceptible when the temperature is above 85° F.

Transmission of a virus from mosaic infected American

elm to peach. MOORE, J. DUAIN, AND E. H. VARNEY. In March, 1952, 2 potted Lovell peach seedlings in the greenhouse were inoculated by chip budding with strips of bark from an American elm infected with elm mosaic virus. In the spring of 1953, these trees were severely dwarfed with compact rosettes of smaller than normal leaves. Individual leaves developed irregular chlorotic areas that later became necrotic. Most necrotic areas fell out, giving a "shredded leaf" effect. The 2 noninoculated controls remained healthy. Mechanical inoculations from peach leaves to Havana 38 tobacco produced the same type of ring spot symptoms that were obtained by direct mechanical inoculations from diseased elm to tobacco. The diseased peach trees again showed symptoms in 1954. However, on 1 tree, 2 shoots were entirely symptomless. Mechanical transmissions to tobacco were obtained as readily from these recovered shoots as from those that continued to show symptoms.

An air blast method for rapid inoculation of wheat and barley with loose smut. MOORE, M. B., AND DONALD E. MUNNECKE. This method permits inoculation of 2–4 heads per minute and in 2 years' tests has resulted in 74 and 90 per cent infection in barley and 58 and 82 per cent infection in wheat. Dry spores are placed in an 8-oz. mayonnaise jar which is attached beneath a mechanic's air dust gun. An adjustable air jet is directed into the jar and raises a cloud of spores, which in turn are entrained by the main air blast of the gun. The air blast, if directed at the spikelets of grain from a distance of 1 in., carries the spores into all parts of the florets and many of them lodge on the stigmas. Air is supplied by a small, portable power compressor. The main jet has an opening 1.6 mm. in diameter. Most satisfactory pressure for inoculating wheat was 60 lb., and for barley, 40 lb.

Control of bacterial halo blight on garden bean in Montana in 1952 and 1953. MORRIS, H. E., AND M. M. AFANASIEV. Topcrop bean seed stock grown in Idaho and free of halo blight (*Pseudomonas phaseolicola* (Burk) Dows.) was planted on new ground. Blight infected beans were planted in rows adjacent to each plot as an inoculum source. Plants were also sprayed with a water suspension of inoculum in the first trifoliate leaf and prebloom stages. Bordeaux 4-4-50, Microgel (basic copper sulfate), and Dithane Z-78 (zineb) 1½-100 were used in 1952. Bordeaux, Microgel, Manzate 1½-100, and Captan 50, 4-100 were used in 1953. One series of plots was sprayed 4 times at 10 day intervals beginning July 10 and a second series twice, July 24 and August 10. In 1952 there were no significant differences between treatments in yield. Four applications of Bordeaux and Microgel gave best control of the disease on leaves, stems, and pods. In 1953, the Bordeaux plot produced one of the best yields and 98 per cent marketable pods with only a trace of disease on the leaves. The other chemicals gave good control on leaves and stems but produced 63–75 per cent marketable pods in comparison to the check which had 56 per cent. Four sprays were distinctly better than 2.

Antagonistic effects of the soil microflora upon sclerotium rolfsii. MORTON, DONALD J. Antagonistic effects of the microflora of Louisiana soils upon *Sclerotium rolfsii* were studied by isolating micro-organisms at random from soils and testing them individually in petri dishes. Of 1,114 bacteria, 2 (0.18 per cent), of 1,064 actinomycetes, 18 (1.69 per cent), and of 1,005 fungi, 35 (3.48 per cent) inhibited growth of the fungus. The antagonistic bacteria and actinomycetes produced an inhibition zone, whereas the fungi overgrew *S. rolfsii.* The 2 bacteria were identified as strains of *Bacillus subtilis;* the 35 fungi all appeared to be *Trichoderma* sp. Several greenhouse tests were conducted with the antagonists and *S. rolfsii,* 3 methods of soil inoculation being used: 1) cornmeal-sand inoculum mixed in soil, 2) liquid inoculum poured on soil, and 3) agar inoculum placed below the surface of soil. Four-in. pots were used, 10 soybean seeds being planted in each. Disease severity was determined by counting healthy seedlings 14 days after planting. The antagonists tested (2 bacteria, 13 actinomycetes, and 2 fungi) were considered to be a representative sample. The fungi in all cases and the actinomycetes in general significantly reduced disease severity, whereas the bacteria had no effect.

Mode of fungicidal action of potassium dialkyldithiocarbamates. MUNGER, G. D., R. W. GREENLEE, AND M. M. BALDWIN. The relationship of sorption to fungicidal activity of potassium dialkyldithiocarbamates was investigated with S^{35}—labeled homologues of contrasting toxicity. The less toxic di-n-propyl compound was more strongly sorbed by spores of *Helminthosporium sativum* and *Monilia fructicola* than the dimethyl homologue. The same relative sorption differences also occurred with dead spores, which, however, sorbed a significantly greater amount of di-n-propyldithiocarbamate. Uptake was proportional to duration of exposure and, in the case of the dimethyl derivative, fungitoxicity. In view of the close chemical similarity and widely divergent toxic properties of these compounds and the results obtained, a possible explanation is that the di-n-propyl compound is strongly adsorbed on the spore wall, preventing permeation of the chemical into the spore; the dimethyl compound, being less strongly adsorbed, enters the cell and reaches the site of action.

Biological assay technic for studying fungicide drenches in soil. MUNNECKE, DONALD E. Rapid, reproducible results on penetration of fungicides have been obtained by drenching soil columns and placing samples of this soil on media seeded with fungus spores. Inhibition zones form, varying with type and concentration of fungicide and depth of sample. Of 6 soil fungi tested, *Myrothecium* sp. was selected, since it grows uniformly, quickly, and inhibition zones are easy to measure and photograph. It can colonize soil, offering an opportunity to determine non-diffusible fungicide effects. Uniform columns were formed by packing a constant weight of soil into a given volume. Solutions of fungicides were applied in quantities 50 per cent greater than that required to saturate the soil. Soil was taken at various levels, packed into plastic rings, placed upon freshly seeded agar plates, and rings removed. Spores were suspended in cooled Difco potato dextrose agar, and 20 ml. added per Petri dish (100 mm. diameter). Measurable zones of inhibition were formed in 48 hours. Analysis showed that 2 samples and soil columns were sufficient to give valid results. The technic is useful in determining the role of soils, concentration of fungicides, leaching, soil organisms, and other factors on the effectiveness of soil drenches.

Etiology and control of black spot of papaya in Hawaii. MURAKISHI, H., M. ARAGAKI, AND H. KAMASAKI. A serious fruit and leaf spot of papaya (*Carica papaya*) occurred during the winter and spring of 1952–53. A *Cercospora* was consistently isolated from the black, crater-like spots on the fruits and from grayish-white subcircular spots on the leaves. The fungus was identified by Dr. Charles Chupp as *Cercospora papayae* Hansford which was previously reported from Africa on leaves. However, there have been no previous reports of a fruit spot of papaya caused by a *Cercospora.* Isolates obtained from both infected leaves and fruits near Hilo were atomized on leaves and fruits and the plants kept under constant fog for 4 days. Typical symptoms were reproduced on both fruits and leaves and the fungus was reisolated. Spray tests on 2 farms near Hilo resulted in good control of the leaf spot phase. On farm A, the descending order of effectiveness was ziram (0.7 spot/leaf), Manzate (0.9), tribasic copper sulfate (2.2), ferbam (3.1), and check (54.8). On farm B, it was zineb (.5), ziram (.6), captan (1.4), tribasic (12.4), and check (118.4).

Comparative studies of six isolates of orchid virus in Hawaii. MURAKISHI, H., AND M. ISHII. Comparative studies on symptomatology, host range, physical properties, cross-protection, and serology were made with isolates of virus obtained from naturally-infected orchids found in Honolulu. Distinctive symptoms of the original source plants were these: *Cattleya* sp. (leaf necrosis), *Cattleya* sp. (leaf

mosaic and flower color break), *Cymbidium* sp. (leaf mosaic), *Dendrobium superbum* (leaf mosaic and flower color break), *Spathoglottis* sp. (diamond leaf spot), and *Vanda* (leaf mosaic and flower color break). Although all the isolates caused varying degrees of mosaic symptoms on *Cymbidium* and *Cattleya* and necrotic patterns on leaves of *Spathoglottis*, the Cattleya color-break isolate caused a distinctive top necrosis of *Spathoglottis*. Physical property tests showed this to be the most stable of the 6 virus isolates. It withstood 72°C. for 10 minutes, was infective in vitro at room temperature for 16 but not 32 days, and had a dilution end-point between 10^{-4} and 10^{-5}. Cross-protection tests with the Cattleya color-break isolate as the challenge virus indicated no immunity was afforded to each group of 5 Spathoglottis plants systemically infected with any of the other isolates. However, serological tests with antisera prepared from each of the 6 isolates indicated a positive precipitin reaction in homologous and heterologous combination with antigens.

Heterocaryosis as a mechanism of variation in rust fungi. NELSON, RICHARD R., AND ROY D. WILCOXSON. Urediospores of 2 or more races of *Puccinia graminis tritici* were mixed manually and dusted on compatible and non-compatible wheat varieties. The resulting generation of rust was transferred to resistant wheat varieties. Ninety-six attempted pairings, involving 24 races or biotypes, were studied on 21 varieties and selections of wheat. Four new biotypes were produced which differed from parent races in pathogenicity and/or color. One biotype, originating from a mixture of races 38 and 56, was highly pathogenic on Khapli, a wheat variety hitherto highly resistant to North American races of *Puccinia graminis tritici*. Since its production, this biotype has had a marked degree of instability, as indicated by a gradual loss of virulence, and after more than 30 generations is almost completely avirulent on Khapli. Dissociation occurs on Khapli, and when isolations were made from single uredia of infection type 1 on Khapli, parental biotypes and biotypes characteristic of both races were recovered. Cytological studies indicate that some urediospores of the virulent biotype are 3-4 nucleate, while urediospores of parent races and recovered biotypes are binucleate.

Variation in the sweet cherry rugose mosaic complex. NYLAND, GEORGE. Five collections of sweet cherry rugose mosaic from different sections of northern California could be placed in 2 groups, based on symptom expression in different hosts and rate of virus movement in inoculated trees. One group consisting of 3 collections caused moderate to severe symptoms the spring following previous summer inoculations in Bing, Napoleon, Black Republican, and Black Tartarian varieties of sweet cherry, Texas and Nonpareil almonds, French Prune, and mild symptoms on Elberta peach. In all hosts except peach, shock symptoms resulted the first year with milder symptoms the following year. Rugose shock symptoms in cherry and almond could be distinguished from those of ringspot. The other group consisting of 2 collections did not induce symptoms on the same hosts until 2 and sometimes 3 years after inoculation. Symptoms first appeared on cherry near the base of the trees and gradually spread upward during the 2 succeeding years the trees were under observation. Only mild symptoms have appeared on almond and peach. Both groups produce a range of symptoms that fit fairly well within the description of sweet cherry rugose mosaic as reported by Thomas in 1939. Certain additional fruit and leaf symptoms should be added to the description.

Northern Peru, a possible new source of potatoes resistant to Phytophthora infestans. OCHOA, C. In 1952 more than 400 samples of cultivated, wild, and weed potatoes were collected in the previously unexplored zone of northern Peru. These are now maintained in the germplasm bank at the Experimental Agricultural Station of Huancayo, Peru. Taxonomic and agronomic studies of the material are in progress and will result in a clearer idea of the distribution of cultivated and wild species as well as their usefulness in potato breeding programs. In tests of resistance to the races 0; 1; 4; 1,4; and 2,4 of *Phytophthora infestans* made in the greenhouse at the Department of Plant Pathology and Botany of the University of Minnesota, *Solanum chiquidenum* Ochoa sp. nov. (inedit) and *S. piurae* Bitter, both from northern Peru, were found to be immune from all 5 races. No resistance to late blight has been reported until the present time in the potato species of Peru, and it is very significant to have found this characteristic in the northern wild species. It is very desirable to make an exhaustive exploration in Ecuador, especially in the southern part where the native potatoes are unknown. It is highly possible that new species highly resistant to late blight would be found.

Means of differentiating atypical isolates of Sclerotinia laxa and S. fructicola. OGAWA, JOSEPH M., AND HARLEY ENGLISH. Typical cultures of *Sclerotinia* (*Monilinia*) *laxa* and *S.* (*M.*) *fructicola* could easily be distinguished on 1.5 per cent potato-dextrose agar. Atypical cultures were impossible to determine as to species when the same technique of identification was used. Such characters as growth rate, sporulation, and lobing of the colony margins were not reliable features for accurate determination of all California isolates. When numerous California brown-rot isolates were studied, hyphal anastomosis of germinating conidia in 0.1 per cent dextrose solution commonly occurred with *S. fructicola*, whereas with *S. laxa* anastomosis was lacking or rare. In diffuse light, sclerotia-like bodies were formed in most of the *S. fructicola* cultures. Root-like projections of hyphae were formed in the *S. laxa* cultures, kept at 4°C, but no such structures were formed in *S. fructicola* cultures held under the same conditions. Species identification of atypical California brown-rot isolates is possible through the use of these procedures.

Effect of Ustilago tritici infection on third internode elongation in resistant and susceptible winter wheat. OHMS, RICHARD E., AND WAYNE M. BEVER. In this preliminary study, Kawvale, C.I. 8180, (resistant) was inoculated with races 1, 3, and 11 of *U. tritici* (Pers.) Rostr.; and Wabash, C.I. 11384, (susceptible) with race 3. Embryo examinations were made after seed were soaked in 5 per cent NaOH, stained and cleared by heating in a nearly saturated aqueous solution of chloral hydrate with 0.01 per cent acid fuchsin, and mounted in lactophenol. For seedling examination, seeds were planted in soil in 12 in. glazed jars at a 3 in. depth in the greenhouse. After 14 days the seedlings were examined for the presence of mycelia by the staining method described above. In Kawvale the percentage of embryo and scutellum infections for race 1 were 48 and 55; for race 3, 59 and 62; for race 11, 75 and 78; and in Wabash for race 3, 62 and 49, respectively. Associated with the scutellum infection was a shortening of the third internode. In Wabash this reduction varied from 50 to 100 per cent. In Kawvale when the third internode was shortened, the fourth internode elongated. In Wabash seedlings showing the reaction, 100 per cent had scutellar and growing point infection. In Kawvale seedlings showing reactions, 100 per cent had scutellar, but no growing point nor fourth node infection. In Wabash and Kawvale 4 and 17 per cent, respectively, had scutellar infection with no reduction in the length of third internode.

Certain mercurial seed treatments do not kill fungi on seed wheat prior to planting. OLIEN, CHARLES R., AND M. B. MOORE. A seed lot was selected in which 99 per cent of the seeds carried internal fungi and more than half of the seeds contained pathogenic species of *Fusarium*. Portions of this seed lot were treated with Panogen (2.2 per cent methyl-mercury-dicyandiamide) at a rate of ¾ fluid oz. per bushel and Setrete (7 per cent phenyl-mercury-ammonium-acetate) at a rate of ½ fluid oz. per bushel. The treated seed was stored in sealed containers for various periods, and then was plated on acid potato-dextrose-agar. Seventy-five per cent of these seeds yielded slow growing colonies of fungi. Sodium thiosulfate has been shown to be effective for reviving microorganisms which have been inhibited by

mercurials. Virtually 100 per cent of seed treated as above and then after-treated with sodium thiosulfate and plated on acid potato-dextrose-agar yielded fungus colonies, and these colonies after 5 days were more than twice the size of those in the preceding test. Evidently Panogen and Setrete inhibit seed borne fungi but do not kill many of them during the time prior to planting the seed.

Variation in Phytophthora palmivora isolated from cacao and rubber. ORELLANA, RODRIGO G. *Phytophthora palmivora* Butl. from *Theobroma cacao* L. and *Hevea brasilienses* (H.B.K.) Muell-Arg. grown separately on potato-dextrose-agar differs in appearance, growth rate, and spore size. Isolates from rubber grow in waves and faster than smooth colonies of isolates from cacao. Sporulation of isolates from rubber is scant and slower with smaller spores than isolates from cacao. In cacao-dextrose-agar, rubber isolates produced few spores, while cacao isolates produced more spores but also abnormal nodulations. Growth of rubber isolates on the basis of dry weight in cacao-dextrose broth was inferior to growth of cacao isolates. In this medium sporulation of rubber isolates did not occur. Cacao isolates inoculated on leaf petioles and terminal buds of rubber stems caused blackening and defoliation. Cacao trees similarly inoculated with rubber isolates did not become infected. Cankers were induced occasionally on woody stems of rubber plants by cacao isolates 3 months after inoculation. No infection of cacao by rubber isolates has been obtained thus far either in laboratory or field. Because of differences in pathogenicity and in colony appearance, growth rate, spore size, and sporulation on artificial media of both isolates, the species *Phytophthora palmivora* Butl. from cacao and rubber should be given variety names according to the host, as *P. palmivora theobromae* and *P. palmivora heveae.*

The morphology and life history of Phyllachora punctum. ORTON, C. R. Ascospores germinate readily in water and synthetic media, developing germ tubes which attain a length 5 times that of the spores and terminate frequently in *thick walled* ovate bodies similar to appresoria. No further development takes place on synthetic media. Fresh ascospores of *Ph. punctum* sown on its *Panicum* host, in the humidity generated under a bell jar lined with moist absorbent paper, produce infection in 16–24 days. Numerous spermagonia develop under the epidermal clypeus, producing spermatia measuring 0.5 × 7.0–10.0 μ which exude through an ostiole to produce delicate spore horns. Ascocarps containing mature asci may develop within 6 to 10 days following the spermagonia, or spermagonial development continues and may extend considerably within the host without ascocarp formation, suggesting this species to be heterothallic. The ascospores are forcibly discharged, usually in pairs, to a vertical distance of 8 mm. for wind dissemination. Reproduction by ascospores continues throughout the summer until the overwintering rosette leaves are formed which become infected and harbor the fungus through the winter in living state. No conidial stage has developed in culture work.

Studies of the cucumber Fusarium wilt fungus. OWEN, JOHN H. Isolates of a Fusarium from stems of wilted cucumbers grew profusely on potato-dextrose-agar, producing a white cottony growth with a pink to purple pigment. Microconidia and chlamydospores were formed abundantly in young cultures with a sparse development of macroconidia occurring in older cultures. An isolate of the fungus has been identified as belonging to the species group *Fusarium oxysporum* (Schl.) Sny. & Han. Young cucumber seedlings are readily infected and killed when grown in steam sterilized soil inoculated with the cucumber Fusarium. No infection occurred when cucumber seedlings were grown in soil inoculated with *Fusarium oxysporum* f. *niveum* (E. F. S.) Sny. & Han. or *F. oxysporum* f. *melonis* (Leach & Cur.) Sny. & Han. Inoculation of soil in pots containing 4-week old cucumber plants with *Fusarium* spp. from cucumber (2 isolates) resulted in 100 per cent kill within 20 days. Similar inoculations with isolates from watermelon and muskmelon did not lead to infection. In field experiments, cucumbers planted on soil infested with cucumber Fusarium developed symptoms of wilt during all stages of growth.

Pathways of metabolism in conidia of Neurospora sitophila. OWENS, ROBERT G. Studies on pathways of glucose and acetate oxidation by conidia of *Neurospora sitophila* indicated that metabolism may differ from that of higher plants and animals. R.Q. values obtained on endogenous respiration of intact conidia were characteristic of fat metabolism. Glucose, acetate, pyruvate, acetoacetate, and lactate greatly stimulated oxygen uptake by intact conidia, whereas acids ordinarily appearing as intermediates in the citric acid cycle had little or no effect on oxygen uptake. The citric acid cycle, therefore, appears not to be the principal pathway of glucose or acetate oxidation. By use of appropriate inhibitors and added cytochrome c, evidence was obtained that a cytochrome system is the terminal oxidase system in acetate metabolism. R.Q. values in the presence of acetate showed that acetate is oxidatively condensed without simultaneous decarboxylation. The condensation product appeared to be acetoacetate. Acetoacetate added to spores was rapidly oxidized and decarboxylated. The decarboxylation product appeared to be methylglyoxal since this compound added to conidia gave the same effect as lactate to which it is readily converted. Lactate greatly increased CO_2 release but not O_2 uptake initially. This suggests that lactate is decarboxylated to yield acetate, thus completing a cycle in which 1 mol. of acetate is consumed.

Effects of fungicides on polyphenol oxidase. OWENS, ROBERT G. Inhibition of the copper-dependent polyphenol oxidase from *Agaricus campestris* L. by several classes of fungicides was tested, and attempts were made to correlate inhibition of the enzyme with fungitoxicity and with chemical makeup of the fungicides. All dithiocarbamic acid derivatives, except copper salts, and all thioureas and thiocarbazides were inhibitory for the enzyme. Inhibition was dependent upon a sulfur atom capable of tautomeric conversion to a sulfhydryl group which could complex with the copper atom of the enzyme and thus bring about its inactivation. Basicity of the amine nitrogen also was important in inhibition and seemed to be related to stability of the enzyme-chemical inhibition complex. Effects of the chemicals on polyphenol oxidase were satisfactorily correlated with fungitoxicity in case of only 1 of 4 fungus species, e.g., *Aspergillus niger.* Quinones and phenolic compounds were noninhibitory or only slightly inhibitory for the enzyme. Protein precipitants such as mercuric chloride were less inhibitory for the enzyme than the better metal complexing agents, indicating that the copper atoms are more accessible at the enzyme surface than are sulfhydryl and other groups subject to attack by protein precipitants.

The fungicidal value of several volatile chlorinated hydrocarbons. OYER, EDWIN B., AND GEORGE A. GRIES. (member A.P.S.). The vapors of chlorinated hydrocarbons have been found to reduce development of brown rot of peaches and Rhizopus leak of strawberries. Chlorinated ethanes and ethylenes were among the most effective and caused little or no damage to the hosts. Dose-response curves were determined for several of these chemicals against conidia of *Monilinia fructicola.* The LD 50 and LD 95 values (mg. per liter of air) of ethanes and ethylenes are in the order of decreasing toxicity: S-tetrachloroethane, 6.4 and 10.8; tetrachloroethylene, 10.5 and 18.0; 1,1,2-trichloroethane, 19.8 and 33.5; and trichloroethylene, 37.5 and 59.0. Dose-response curves are nearly parallel suggesting a similar mode of action for all chemicals. No further germination occurred after removal of high concentrations of tetrachloroethylene indicating that the materials are fungicidal, not fungistatic. In studying time of exposure as the dose and holding concentration constant, straight line dose-response curves are obtained but the slope is steeper with higher concentration. Mycelium of *M. fructicola* is more sensitive to tetrachloroethylene than

germinating spores. There was only a slight increase in colony diameter following treatment at the rate of 5 mg. per liter of air and no growth at slightly higher concentrations.

A modified plating technique for the study of soil fungi. PAHARIA, K. D., AND T. KOMMEDAHL. Despite the fact that dilution technique does not give a true picture of soil microflora, it has been extensively used for both quantitative and qualitative determinations of soil fungi. In the present work, 2 methods of plating on 15 different media were compared with respect to numbers and kinds of fungi isolated. Distributing 1 ml. of soil dilution over the solidified agar surface 2–3 days after plates were poured was compared to incorporating the soil dilution in the medium at the time of pouring plates. On most media, but not all, the former method resulted in 2–3 times as many fungus colonies per gm. of oven-dried soil as the latter. Addition of rosebengal, as a bactericidal agent, to a medium resulted in statistically significant increases in the number of colonies as compared to the addition of acid or salt. Highly significant increases in colony numbers occurred if both streptomycin and rosebengal were added to a medium. The kinds of fungi isolated as well as the numbers varied with the medium used. Species of *Fusarium, Pythium, Cephalosporium, Verticillium,* and *Alternaria* were favored by the addition of rosebengal or rosebengal plus streptomycin but were suppressed by acid and salt. Species of *Rhizopus, Mucor, Aspergillus, Penicillium,* and *Trichoderma* grew well on all media.

Oak wilt development in bur oaks. PARMETER, J. R., AND J. E. KUNTZ. Bur oaks inoculated with *Endoconidiophora fagacearum* Bretz commonly showed wilting leaves on scattered branches and lived for several years with progressive stagheading. Symptomatic tyloses, which plugged the xylem vessels, were limited to a narrow, vertical sector of the bole and to the vascularly connected branches. Foliage wilt subsequently developed on these branches. The wilt fungus could be isolated only from such trunk sectors or branches. Dyes and radioactive isotopes did not move through the plugged vascular tissue but did move freely throughout the rest of the tree. During the second season, new wood usually formed over the infected, plugged sector and symptoms appeared sporadically during the summer. Sometimes, no foliage symptoms appeared the second season. Root grafts between adjacent bur oaks were rare. Only 1 root graft between a bur and a northern pin oak has been detected. In mixed stands, bur oaks often escaped infection while adjacent red and black oaks died. Fungus mats have not been found on infected bur oaks even at times when they were abundant on neighboring northern pin oaks.

The effect of air temperature on incidence and development of the gray leaf spot and nailhead spot diseases of tomato. PAULUS, A. O., AND G. S. POUND. Disease surveys in 1952 and 1953 failed to reveal the occurrence of either *Stemphylium solani* or *Alternaria tomato* in Wisconsin. Spore germination and growth in pure culture for both fungi occurred over a temperature range of 4°–36°C. In greenhouse infection studies, using a temperature range of 10°–26°, the most infection with both pathogens was obtained at 26° and the least at 10°. Infection with *S. solani* was obtained after 3 hours at 14° and 26° but only an occasional lesion at 10° even after 48 hours. Disease development was studied at temperatures of 16°, 20°, 24°, 28°C. With dilute inoculum of the gray leaf spot fungus, disease development was very slight at 28° and moderate to severe at 16°. With concentrated inoculum, development was equally severe at 16°, 20°, and 24° but markedly reduced at 28°C. With nailhead spot, complete defoliation was always obtained whether inoculum used was dilute or concentrated. High temperatures merely hastened defoliation. Keeping plants infected with *S. solani* for 12 hours at 16° and 12 hours at 28° did not increase disease development over those plants held constantly at 16°. Sporulation of *S. solani* on infected plants was practically nil at 10°C., but with *A. tomato* sporulation occurred at this temperature 36–48 hours after placing infected plants in a moist chamber.

Ascorbic acid content of stem-rust resistant and susceptible wheats. PILGRIM, A. J., AND M. C. FUTRELL. Experiments to determine whether ascorbic acid functions in resistance of wheats to *Puccinia graminis tritici* have been conducted. Four tetraploid wheats and 5 hexaploid wheats were analyzed for Vitamin C content at 8 growth stages. Varieties analyzed showed marked differences in ascorbic acid content at different growth stages. Khapli, resistant to many biotypes, had the highest Vitamin C content at tillering (220 mg. expressed on basis of 100 gm. fresh weight). Hard Federation, susceptible to many biotypes, had 145 mg. In the fast growing stage, late tiller to flowering, resistant varieties St. 464, Khapli, and Wisconsin 245 showed constant Vitamin C contents (90 mg.). Susceptible Hard Federation dropped slowly from 145 mg. at tillering to 76 mg. at boot stage. Ascorbic acid content of resistant Kenya 338-AC-2-E-2 dropped sharply in the joint stage to 47 mg., but tended to stabilize near 85 mg. from boot to flower. Yaroslav and Hope varieties declined in ascorbic acid content from tiller to flower stage, but increased from flower to soft dough stage. The mature plant resistance of Hope functions at this growth stage. In the greenhouse Hope showed very little variation in Vitamin C content at 65° and 80°F. Other varieties showed higher amounts at 65°F.

Histological and cytological studies of plants infected with cucumber mosaic virus. PORTER, CLARK A. A study was made of the histological and cytological changes induced by cucumber mosaic virus in cucumber, Clara Butt tulip, and broad bean and by the necrotic fleck complex (cucumber mosaic virus in combination with lily symptomless virus) in Croft Easter lily. In all cases, the initial symptoms originate in the mesophyll of affected leaves. Symptoms are confined to this tissue in mottled cucumber leaves but the necroses produced in the other species ultimately involve other tissues. The yellow areas in young, mottled cucumber leaves appear hyperplastic due to differentiation proceeding more rapidly than in comparable healthy leaves. However, the yellow areas in mature leaves are hypoplastic. Necrosis in Easter lily and tulip is largely restricted to the mesophyll of infected leaves and, in tulip, is associated with enlargement of cells and cell wall thickening. In both plants, vascular tissues become necrotic only after severe degeneration of the mesophyll. In contrast to this, the necrosis in broad bean rapidly progresses from the mesophyll to vascular tissues.

Relationship between timing of bactericidal applications and spring canker development on peaches. POWELL, DWIGHT, ANGELOS V. ADAM, AND H. W. ANDERSON. A 2-year-old block of 140 Elberta peach trees was divided into randomized, single-tree plots replicated 5 times. Forced-stream inoculations with *Xanthomonas pruni* were made on all trees on July 25, August 12 and 20. Ten days after the third inoculation, foliage infection averaged 74 per cent. The following were compared for abundance of 1954 spring cankers associated with primary infection: a) Copper sulfate, 1 lb./100, b) Hyamine 1622, 4 oz./100, and c) Neomycin, 2 oz./100. Triton B1956 3 oz./100 was used with each material. Sprays were applied in 1953 as follows: Plots 1a, 1b, 1c, August 1; Plots 2a, 2b, 2c, August 1, 16; Plots 3a, 3b, 3c, August 1, 16, September 4; Plots 4a, 4b, 4c, August 16, September 4, 18; Plots 5a, 5b, 5c, September 4, 18, October 2; Plots 6a, 6b, 6c, September 18, October 2, 16. The bactericides manifested a similar pattern of control of spring cankers depending on time of application. On June 16, 1954, there was an average of 13 cankers per tree on the unsprayed check trees. Plots 3 and 4 ranged from 0 to 1.8 cankers per tree. The remaining plots showed an average of 4–10 cankers per tree. Thus, most effective control was obtained in a series of 3 sprays either on August 1, 16, and September 4 (Plot 3), or on August

16, September 4, and 18 (Plot 4). Phytotoxicity was negligible in all plots.

Differential reaction of potato hosts to foreign and domestic physiologic potato races of Phytophthora infestans. PRISTOU, R., AND M. E. GALLEGLY. Differential potato hosts and potato physiologic races of *P. infestans* reported by Black in Scotland and Mastenbroek in the Netherlands were brought together for comparisons with the United States hosts and races of Mills and Peterson. Included in the tests were 3 isolates from Canada. The differential hosts contained either 1 of 4 genes (R_1, R_2, R_3, and R_4) or combinations of them. The physiologic races and genes for resistance reported by the above workers fell into a definite pattern. The results confirm the interrelationship of genes and races reported jointly by the above workers, and show that their proposed international system of designating these genes and races is valid and workable at present. Isolates of the above workers fell into 10 groups matching the international races 0; 1; 2; 4; 1,2; 1,4; 2,4; 1,2,4; 1,3,4; and 2,3,4. The Canadian isolates tentatively identified by Black were found to be races 1,2,3: 1,2,4 and 1,2,3,4. These 12 different races, together with 3 others (3; 1,3 and 3,4), described by Black but not available for study, bring the total number of races found to 15. Only race 2,3 is missing from the 16 races identifiable with the present differential hosts.

Differences in phytotoxic properties exhibited on spring wheat by fungicides used for smut control. PURDY, LAURENCE H., JR., AND C. S. HOLTON. Tests with Panogen (methylmercury-dicyanidamid 2.2 per cent) in 1953 indicated a low order of phytotoxicity in this material when used to treat seed for wheat smut control. Good stands were obtained from seed treated with increasing dosages up to 5 oz. per bu. At higher dosages stands were reduced but not eliminated, even at 10 oz. Similar results were obtained in 1954 in tests which included Panogen, Ceresan M (ethyl mercury p-tolnene sulfonanilide 7.7 per cent), Agrox (phenol mercury urea 6.7 per cent), and Anticarie on 4 spring wheats. The materials were applied to Baart, Idaed, Federation and Marfed in dosages of ½, 1, 2, 3, 4, 5, 8, and 10 oz. per bu. Differences in phytotoxic properties of some of these materials were apparent, especially at rates above 5 oz. At minimum dosages required for smut control all materials were slightly and about equally phytotoxic. Striking differences in phytotoxicity occurred in overdosages. The maximum permissable dosage for Agrox and Ceresan M was 1 oz. per bu. and for Panogen 2 oz. per bu. At higher dosages Panogen consistently was less phytotoxic than Agrox and Ceresan M. The slight phytotoxicity exhibited by Anticarie at ½ oz. per bu. did not increase with higher dosages. Slightly greater tolerance to phytotoxic action of fungicides was exhibited by Marfed and Baart than by Idaed and Federation.

Inactivation of tobacco mosaic virus with rabbit antisera. RAPPAPORT, IRVING, AND ALBERT SIEGEL. Rabbit antiserum reduced the infectivity of the common strain of TMV (U1) according to a law of multiple proportions. The log percentage of virus activity remaining after reaction of 5×10^{-4} mg. virus protein/ml with antiserum, was directly proportional to the log of the serum concentration within the dilution range of 10^{-5} ml. to 10^{-3} ml. Infectivity was lost very rapidly. A dilution of 5×10^{-4} ml antiserum lowered the activity of 5×10^{-4} mg. virus to less than half the initial value in 30 seconds. Antibody-virus aggregates were demonstrated by centrifuging serum-virus mixtures at moderate centrifugal forces and comparing the infectivities before and after centrifuging. Aggregation increased with increasing concentrations of the reactants. The fraction of virus activity surviving after treatment with antiserum was independent of the reactant concentrations but was a function of the antiserum-virus ratio at the time of mixing. Ultraviolet inactivation curves of antibody-virus aggregates suggested that those aggregates which retained infectivity contained no more than 1 viable virus particle. These results in conjunction with the centrifuge data suggested that only a minute fraction of the total virus population as ordinarily prepared was capable of initiating an infection.

An association of late-breaking virus in potato with a phyllody condition in Ladino clover. RAYMER, W. B., AND CLARK R. AMEN. An unusual disease of potato appeared in central Oregon in 1946 and was described as the late-breaking virus disease. In many fields, infection ranged from a trace to 3 per cent. In 1950, a marked increase in prevalence of the disease occurred coincident with an increase in acreage of Ladino clover. A 1953 survey showed an average disease incidence of 12 per cent, with individual fields as high as 36 per cent. A phyllody condition was general in adjacent Ladino clover fields. Further investigation revealed a proliferation of lateral buds and phyllody in species of *Lactuca, Brassica,* and *Erigeron* growing as weeds. Aster leafhoppers from these fields were fed on China asters, celery, lettuce, and White Rose potatoes. Aster yellows symptoms were produced on all but the latter, which showed symptoms described for the late-breaking virus. This virus appears to be a strain of the aster yellows complex. Field spread of the disease is correlated with movement of the leafhoppers onto the emerging potatoes when clover is clipped in the spring. A hairsprout condition consistently associated with the disease has not been found associated with other potato diseases in Oregon.

Some physical biochemical characteristics of squash mosaic virus. RICE, ROBERT V., MARK A. STAHMANN, GEORGE D. LINDBERG, AND J. C. WALKER. This virus when isolated from small sugar pumpkin plants by a combination of acid precipitation and differential centrifugation (precipitation at pH 5.0 and subsequent centrifugations), showed 3 components in the ultracentrifuge, but only 1 schlieren peak upon electrophoresis at 4 pH values (pH 4.75, 6.05, 7.50, and 8.25). The isoelectric point was 4.65 at 0.10 ionic strength, and the sedimentation coefficients were 111 S, 88 S, and 56 S (S = Svedberg units). The relative amounts of the components were constant (34, 62, and 4 per cent respectively) when isolated from plants infected from 1 to 8 weeks. The "whole" virus system showed a maximum ultraviolet absorption at 2585 A, a minimum at 2400 A, an inflection at 2900 A, and a ratio of optical density at 2585 to 2800 of 1.79. The top component (56 S) obtained by the use of an ultracentrifuge separation cell was infectious and had a maximum at 2575 A, a minimum at 2370 A, and a 2575/2800 ratio of 2.08. Some of the particles in this virus system appeared as polyhedrons under the electron microscope when prepared by the freeze drying method. Possible explanations for the existence of the 3 components in this virus system will be discussed.

Control of Botrytis sp. on stored roses. RICH, SAUL. In 1953, a series of fungicides was tested for the control of *Botrytis sp.* on dormant roses in storage. The treatments were applied but once when the roses went into storage. The plants were stored for 2 months at 35–45°F. and 80 per cent relative humidity. Only pentachloronitrobenzene as a 20 per cent dust inhibited the growth of the fungus. The following materials were ineffective: thiolutin, dichlone, chloranil, Manzate, Actidione, zineb, ferbam, Iscothan, sodium dehydroacetate, captan, ziram, Natriphene, and certain other chlorinated nitrobenzenes. All chlorinated nitrobenzenes not only reduced sprouting of the roses in storage, but also retarded the breaking of dormancy when the treated bushes were set out. In 1954, the only fungicide tried was a 1 per cent spray of Vancide 51. The stored roses were sprayed every 2 weeks for a total of 5 applications. The experiment included over 2000 plants and 14 different varieties, stacked in a commercial rose storage house. At the end of the storage season, 44 per cent of the unsprayed checks showed active *Botrytis* growth, as compared to 5 per cent of the sprayed plants. Treated plants showed no spray damage when set out in the field.

Seedling chemotherapy for the control of lettuce big vein. RICH, SAUL. Chemotherapeutants were applied to Penn

Lake lettuce seedlings in flats prior to setting the plants out in a field infested with the virus of lettuce big vein. Flats were watered twice weekly with 500 ml. of each treatment for a total of 6 applications. The plants received no chemotherapeutants after being set in the field. Plants treated with the following had significantly less big vein than the water check: naphthaleneacetic acid (50 and 100 p.p.m.), 2,4,6-trichlorophenoxyacetic acid (50 p.p.m.), indoleacetic acid (50 p.p.m.), calcium chloride (1000 p.p.m.), and zinc sulfate (250 p.p.m.) The first 2 compounds named were significantly more effective than the last 3. CC 1182 (100 p.p.m.) and Orthocide 406 (captan) (2500 p.p.m.) were ineffective against big vein. Napthaleneacetic acid and CC 1182 significantly reduced the weight yields, indicating phytotoxicity at the concentrations used. Only zinc sulfate gave weight yields significantly higher than the check. The increased yield with zinc sulfate may have resulted from remedying an undetected zinc deficiency as well as alleviating big vein symptoms.

Infection of rye by Puccinia graminis avenae. ROBERTS, B. J., AND F. K. S. KOO. It has been presumed that rye, which is heterozygous, is resistant to *Puccinia graminis avenae.* However, when Florida Black was inoculated with race 8 and then grown at 85°F. a few plants were susceptible. This rust was increased on the oat variety Bond and inoculated onto the rye varieties Emerald, Adams, Dakold 23, Florida Black, Horton, Storm, Kings II, Imperial, Dominant, Von Runker, Caribou, Tetra Petkus, and Canadian Spring. A second set of rye varieties was inoculated with race 7. In every variety at least a few plants were susceptible to race 7 or 8 or both. When the varieties were inoculated with either race 7 or 8 and then grown at 75°F., the reaction of the plants ranged from immunity to slight susceptibility and there were only a few slightly susceptible plants. When inoculated and then grown at 85°F., the reaction of plants ranged from immunity to high susceptibility and there was a greater number of susceptible plants in each variety.

Relative concentration of potato virus X in double and single infections. ROCHOW, W. F., AND A. FRANK ROSS. The ratio of the amount of virus X (mild strain) in juice from Turkish tobacco plants doubly infected with virus X and an unrelated virus, to that in juice from comparable plants infected only with virus X was determined by lesions on plants of *Gomphrena globosa.* When leaves were sampled in the acute stage of infection, this ratio reached 6:1 with tobacco etch virus and 2:1 with cucumber mosaic virus. Infections with alfalfa mosaic virus did not result in a change in virus X concentration, but infections with tobacco ringspot virus resulted in as much as a 50 per cent decrease in virus X concentration. At the dilutions used, these 4 viruses did not interfere with biological assay of virus X. In combination with tobacco mosaic virus, virus X could be assayed only by serological methods. Precipitin tests showed at least a 4-fold increase in virus X antigen in this double infection. The concentration of tobacco mosaic virus was unaffected or slightly reduced by virus X as shown by lesions on X-infected plants of *Nicotiana glutinosa.* In general, when the concentration of virus X increased in double infections, the increase was correlated with an increase in severity of symptoms.

New germ plasm for combined resistance to Helminthosporium blight and races 45, 101, and allied races of crown rust of oats. ROSEN, H. R. Because of the danger of all oat breeders using the same germ plasm for combined resistance to Helminthosporium blight and race 45 and allied races of crown rust, a prolonged search for additional germ plasm offering similar resistance has been under way since 1945. In 1946 a single rust-free plant was discovered in a relatively large field plot at the University farm, Fayetteville, Arkansas, the plot being heavily rusted with race 45. Morphologically, the only distinguishing character observed between this plant and the others in the plot was the production of supernumerary florets in some of the upper spikelets. The parentage of this was Tenn. 1922 $\times$ Bond-Iogold, which by itself offered no high degree of resistance to race 45. The single rust-free plant could have originated either as an outcross or as a mutation. The latter appears to be the proper explanation. Many of the selections derived from it show an immune reaction to races 45, 57, and 101 under field conditions during most of the growing season, as well as a high degree of resistance to Helminthosporium blight. Under greenhouse conditions with mean temperatures of about 70–75°F., the resistance to crown rust is almost wholly lost.

Distribution, spread and intensity of dwarf mistletoe on Ponderosa pine. ROTH, LEWIS F. Pine dwarf mistletoe is being studied in central Oregon as a basis for control experiments and stand management recommendations. On 640 acres studied, dwarf mistletoe has a patchwork distribution, in part related to slope, exposure, fire history, and stand structure. Two hundred sixty acres are infested. Of this area, 58 per cent is very light, 29 per cent light, 12 per cent intermediate, and 1 per cent heavy to very heavy. Infestations designated light or greater appear economically damaging. Infected areas on slopes of 0–10, 10–20, 20–30 per cent are 24, 99, and 95 acres or 13, 37, and 76 per cent respectively. Intensity also increases with steeper slope and is greatest on the northwest and least on the northeast exposure. Seeds were dispersed around an isolated overstory tree in an ellipse with maximum and minimum diameters of 185 and 118 ft. This potential spread exceeds spread around other isolated overstory trees where the limits were measured by infection in the understory and suggests very limited spread from tree to tree through the understory. Limited lateral spread in the understory from an infested area outward was further indicated by seed traps placed 6.6 ft. within the infested area, at its perimeter, and at 6.6 and 13.2 ft. outside, that caught 94, 64, 6, and 1 seeds respectively.

Functional role of factors for sexual compatibility in haploid lines of Ustilago zeae. ROWELL, J. B. Corn inoculation experiments demonstrated that tetrapolar sex groups of *Ustilago zeae* result from segregation of 2 pairs of factors, one with 2 alleles (a^1 and a^2) and the other with multiple alleles (b^1, b^2, b^3, etc.). The functional role of these compatibility factors was identified by observations of the behavior between individual sporidia paired by micromanipulation on drops of dilute corn-coleoptile-extract agar. In most compatible combinations sporidial fusions were observed within 3–4 hours and infection hyphae developed within 6–8 hours after mating. Infection hyphae were found to be dicaryotic by the reisolation of both parental lines from cultures of single hyphal tips. Fusions were observed between sporidia of all a^1 and a^2 lines paired but never between sporidia of lines having the same *a* alleles, and these factors apparently govern sporidial fusion. Infection hyphae that developed from fused sporidia having different *b* alleles (ex. $a^1b^2 \times a^2b^3$) were straight, grew rapidly, branched rarely, and produced aerial, haploid conidia only after approximately 24 hours. These hyphae were readily distinguished from hyphae that developed from fused sporidia having the same *b* alleles (ex. $a^1b^2 \times a^2b^2$) which were sinuous, grew slowly, branched frequently, and produced aerial, haploid conidia within 12 hours. Thus, the *b* locus governs vigor and stability of the dicaryon and apparently compatibility of the paired nuclei. (This research was supported in part by a grant from the U. S. Atomic Energy Commission, Contract No. AT (11-1)-42.)

Ethyl mercury p-toluene sulfonanilide, a chemical for control of snow mold of wheat. RYKER, T. C., AND E. F. CLEMENT. Snow mold, caused by *Typhula* sp. and to a lesser degree by *Fusarium nivale,* is a serious disease of wheat in localized areas in the Northwest. Following the work of Roderick Sprague of the Wenatchee, Washington, Station, ethyl mercury p-toluene sulfonanilide (EMTS) was applied in early November, 1953, to large plots on 4 farms in the Fairfield area and in 2 locations in the Tetonia area. The material was applied a) dry at 5 lb. of Ceresan

M (7.7 per cent EMTS) per acre with urea fertilizer compound (NuGreen) as a carrier and b) as a spray at 2½ lb. of Ceresan M-2X (15.4 per cent EMTS) per acre with and without NuGreen. Evaluations were made in early May. Severity of disease varied from severe leaf injury, with plant recovery, where the snow melted early, to practically 100 per cent kill where snow coverage remained relatively late. Spray applications of Ceresan M-2X were superior to dry applications of Ceresan M, and gave essentially 100 per cent disease control under the most severe disease conditions. NuGreen did not aid in disease control but did hasten recovery of injured plants. The EMTS, applied either as Ceresan M or as Ceresan M-2X, did not show any phytotoxic effects.

Effect of bacterial inhibitors on the isolation of fungi by the soil dilution plate technique. SCHMITTEHENNER, A. F., AND G. L. REIGELMAN. As part of a study of the survival of root rot and associated fungi in the soil, the effect of bacterial inhibitors on the isolation of fungi by the soil dilution plate technique has been investigated. The following groups of inhibitors were found to control bacteria: lactic acid to pH 4.5; lactic acid to pH 5.0 with 0.1 per cent oxgall; Chloromycetin 30 p.p.m. and streptomycin sulphate 30 p.p.m.; Chloromycetin 30 p.p.m., streptomycin sulphate 30 p.p.m.; and 1.0 per cent oxgall; rose bengal 30 p.p.m. and streptomycin sulphate 30 p.p.m.; crystal violet 10 p.p.m., streptomycin sulphate 30 p.p.m., and 1.5 per cent oxgall. The first 4 groups were used in yeast extract-glucose-nitrate media, and the last 2 in Martin's and Littman's media, respectively. In the above combinations oxgall decreased the rate of spread of most fungi isolated. In tests with paired samples of corn, wheat, oat, and alfalfa soils, there were no consistent differences among the inhibitor groups with respect to total numbers of fungi per plate and proportions per plate of the most frequently occurring genera of fungi. In these experiments, none of the 6 inhibitor groups appeared selective against any of the soil fungi isolated.

A new concept of resistance to scab of wheat caused by Gibberella zeae. SCHROEDER, HARRY W. Seven varieties of common bread wheats were grown simultaneously in greenhouses under uniform conditions and inoculated with the scab fungus, *Gibberella zeae* during fall and winter of 1953–54. Spores from 5 pathogenic isolates were mixed and suspended in water to inoculate single wheat spikelets by means of a hypodermic syringe or to spray wheat heads by means of a hand atomizer. Wheats were inoculated at the flowering and post-flowering stages. Disease reaction and disease resistance were measured by determining the percentage of normal (non-scabby and non-shrivelled) seed produced. After single-spikelet inoculation at flowering, Thatcher produced 1 per cent normal seed, Rival 83 per cent, and Frontana 79 per cent. After similar inoculation at post-flowering stage, Thatcher produced 53 per cent normal seed, Rival 85 per cent, and Frontana 82 per cent. When wheat heads were sprayed with inoculum at flowering time, Thatcher produced 3 per cent normal seed, Rival 68 per cent, and Frontana 87 per cent. When heads were sprayed at post-flowering stage, Thatcher produced 13 per cent normal seed, Rival 29 per cent, and Frontana 87 per cent. Resistance to scab thus may be of 2 types: a) resistance to infection or b) resistance to the spread of the organism from the point of initial infection.

A rapid tenacity measurement for fungicides using artificial rain and the greenhouse tomato disease method. SCHULDT, PAUL H. One of the important factors in weathering fungicidal deposits is rain. The following technique measures resistance of fungicides against washing from tomato leaves. After tomato plants are sprayed with fungicide and dried, but before exposure to *Alternaria solani* (Ell. & Mart.) Jones & Grout, they are subjected to heavy artificial rain by mounting directly over the plants on opposite sides of a turntable 2 "Teejet" nozzles, No. 6520, with flat spray patterns so that plants pass in and out of rain. The system delivers ¾ in. per minute, measured by a rain gauge on the turntable. Plastic "raincoats" placed over the pots and secured to plant stems keep soil from being over-watered, thereby preventing large increases in lesion counts on plants supersaturated with water. Dosage-response curves of all fungicides tested are parallel on exposed and unexposed plants indicating that the amount of fungicide washed from the leaves is proportional irrespective of dosage. With this established, the relative resistance of fungicides to washing can be determined by spraying a replicated series of plants at a dosage slightly above the ED95 value and subjecting them to no rain or increasing amounts of artificial rain.

Botryosphaeria canker of apple. SHAY, J. R. AND WAYNE R. SITTERLY. Fruit rot caused by *Botryosphaeria ribis* G & D was severe at harvest in southwestern Indiana in 1950 and 1951. The canker phase was not observed until 1952. In July, blisters or wet lesions as much as 6 in. in diameter appeared on main scaffold branches of Gallia Beauty. These invariably yielded the imperfect *Dothiorella* stage upon culturing. By midsummer 1953, cankers were widespread in occurrence, resulting in loss of main scaffolds on formerly highly productive Gallia trees. Deep lesions, ½ in. or more in diameter, caused twig and limb dieback, especially on lower branches. Drought appeared to favor extreme development of the disease. Inoculations of trunks of young trees in the greenhouse were successful only when the trees were subjected to severe drought. A survey of a number of orchards revealed *Botryosphaeria* cankers on practically all apple varieties from southern to extreme northern Indiana. Rome Beauty and Gallia Beauty seem to be most susceptible but severe cases have been found on Cortland, Grimes, and Golden Delicious. Typical *Dothiorella* pycnidia with mature spores were first found on naturally infected limbs in early April, 1954. No ascospores have yet been observed in nature or in culture. Unless checked, the disease may become a major threat to apple production in Indiana.

Breeding poplars for disease resistance. SHEA, K. R., AND A. J. RIKER. In breeding poplars for disease resistance, several problems were encountered and overcome. Flowering was stimulated in dense stands of adult *Populus grandidentata* by trunk girdling, i.e., complete, spiral, and overlapping half-girdles, and by frilling or bruising around the bole. Indoors, floral development on detached poplar branches was favored by using 4-ft. branches with the leaf buds removed, by storage at 20°C. at relative humidities above 40 per cent, by frequent trimming of the branch butts, and by regular water changes. Aspen seeds remained more than 75 per cent germinable after 18 months' storage at 4°C. and approximately 1 per cent relative humidity. Seedling damping-off was circumvented by using a pulverized sphagnum moss substrate. Soil drenches with formalin or with boiling water also controlled the disease. Since 1952, over 4,000 hybrid seedlings have shown striking differences in growth rates, branching, leaf types, tree form, and susceptibility to diseases, insects, and rodents. A native selection (Wisconsin No. 5) grew 41 ft. high and 6 in. in diameter in 6 years. Weed competition in test plantings was reduced by chemical herbicides.

A difference in the infectivity-dilution curves among strains of tobacco mosaic virus. SIEGEL, ALBERT, AND SAM G. WILDMAN. Eight strains of tobacco mosaic virus have been classified into 4 groups on the basis of their electrophoretic mobility, affinity for a host nucleoprotein, sensitivity to inactivation with ultra-violet light, UV absorption spectra, serological properties, and differential response on *Nicotiana sylvestris*. The strains within a group behave similarly as judged by the above criteria. Among groups, differences are found in 2 or more properties. In addition, a difference in biological behavior on *N. glutinosa* has been found since group 1 and group 2 strains yield different infectivity-dilution curves. The virus was suspended in M/15 phosphate buffer, pH 7, containing 50 mg. celite per ml. In the concentration range, 1.56 to 100×10^{-5} mg. virus protein per ml., the logarithm of the number of le-

sions produced by the group 1 strains was found to be equal to 0.67 times the logarithm of the concentration of applied virus. The group 2 strains yield a curve which is more in conformity with that predicted from the Poisson distribution.

Antibiotics tested for control of wheat stem rust. SILVERMAN, WILLIAM B., AND HELEN HART. Various antibiotics dissolved in water with 1 per cent Tween-20 were sprayed on the foliage of Marquis wheat seedlings inoculated 3 days previously with race 56 of *Puccinia graminis tritici*. Only Actidione at concentrations of 20-25 p.p.m. controlled the rust by reducing the number of pustules and by delaying sporulation; at this concentration there was severe dieback of the seedling leaf tips. A saturated water solution of Thiolutin (ca. 120 p.p.m.) had no effect on the rust or the wheat. Crude Terramycin HCl at 600 p.p.m. had no effect on the rust, but caused severe stunting of the wheat seedlings. The following antibiotics did not affect the rust when sprayed on the foliage but caused varying degrees of dieback of the wheat seedling leaf tips: at 500 p.p.m., Dihydrostreptomycin sulfate, Streptomycin calcium chloride complex, Streptomycin formulation STS, Pleocidin, Tyrothricin, Neomycin, alpha-methyl-glutamic acid, Candicidin, MK-65, MK-77, and Streptothricin hydrochloride; at 600 p.p.m., Vet. Streptomycin sulfate and Bacitracin. Soil applications of the antibiotics at the same concentrations did no damage to the wheat nor did they affect the rust.

Inheritance of resistance to common cucumber mosaic virus in cowpea. SINCLAIR, J. B., AND J. C. WALKER. The common strain of Black cowpea is resistant to the common strain of the virus, responding to inoculation by formation of local lesions, whereas a mutant line of Black is susceptible, responding to inoculation by systemic mottle. The susceptible mutant was crossed with resistant Black, Black Eye, and Dixie Queen. All F_1 plants resulting from these crosses were resistant. F_2 progenies segregated in a ratio of 3 resistant to 1 susceptible. In tests for heterogeneity χ^2 for each cross was highly significant, the sum of the total $\chi^2 = 9.2196$ was highly significant, and the sum of the pooled data, $\chi^2 = 0.2695$ was highly significant. F_3 families showed a distribution close to that of 1 homozygous resistant: 2 heterozygous: 1 homozygous susceptible. The χ^2 for each F_3 family was highly significant and the sum of the total of $\chi^2 = 0.9500$ was highly significant. These data show a single dominant gene governing the local-lesion resistant reaction. Twenty-five approach grafts were made between resistant and susceptible plants and the susceptible plant later inoculated with the virus. The resistant plants showed no sign of necrosis and none died during the experiments.

Metabolic processes affected by various fungitoxic compounds. SISLER, HUGH D., CARROLL E. COX, AND PAUL E. HOCHSTEIN. Several fungitoxic compounds were studied to determine whether they inhibit growth of conidia of *Fusarium roseum* Lk. principally by interfering with energy-yielding processes (e.g., glucose degradation), energy-transfer processes, or energy-utilizing processes (e.g., protein synthesis). Concentrations of actidione, thiolutin, chloranil, dichlone, and glyodine which inhibit respiration of growing conidia oxidizing glucose in a nitrogen sufficient medium also inhibit respiration of non-growing cells oxidizing glucose in a nitrogen deficient medium. Growth of the cells is also inhibited by the same range of concentrations of the compounds (for glyodine no growth measurements were made) that inhibit respiration. These compounds apparently interfere with energy-yielding systems or with energy-transfer reactions. Sulfanilamide does not inhibit respiration of glucose by non-growing cells, but does inhibit growth and acceleration in respiration which are associated with utilization of nitrogen. This compound apparently affects energy-utilizing processes. Respiration of non-growing cells oxidizing glucose is stimulated by azide and is approximately equivalent to that of azide-treated cells in a nitrogen sufficient medium. The latter is considerably lower than that of untreated, growing cells. Growth is strongly inhibited. Azide apparently affects growth of these fungus cells by interfering with energy-transfer processes.

Hosts in relation to the incidence of wheat streak mosaic in Alberta. SLYKHUIS, JOHN T. *Aceria tulipae* Keifer transmitted wheat streak mosaic virus (*Marmor virgatum* McK.) to 10 species of wild and 12 species of cultivated annual grasses that can also be infected manually. Sparse populations of mites survived on barley, rye, several wild annual grasses, and the perennial grasses *Poa compressa* L. and *Oryzopsis hymenoides* (Roem. & Schult.) Ricker, all of which can be infected with the virus. *A. tulipae* was found in nature on *Hordeum jubatum* L., *Agropyron smithii* Rydb., and *Elymus canadensis* L., but none of these mites survived on wheat. Similarly, attempts to rear *A. tulipae* from wheat on these grasses failed. Wheat was the only grass tested that was highly susceptible to the virus and readily colonized by *A. tulipae* from diseased wheat. Winter wheat is of predominant importance as an overwintering host for the virus and the vector. Immature wheat plants nurture the virus and the mites during the spring, summer, and fall and thereby supply a potent source of infection for wheat sown in the same or nearby fields. Such sources should be eliminated before sowing wheat in the vicinity. No native grasses have proved important as sources of wheat streak mosaic in Alberta fields.

Problems encountered when spring wheat is seeded after working up streak mosaic diseased winter wheat. SLYKHUIS, J. T., AND J. S. HORRICKS. In southern Alberta, winter wheat is sometimes so severely damaged with wheat streak mosaic that it is advisable to work it up and sow spring wheat. This spring wheat crop has not always been successful due to infection with streak mosaic and to other factors which result in sparse, unthrifty stands. Infection with streak mosaic may result from a poor kill of diseased winter wheat or from seeding before the winter wheat is sufficiently desiccated or decomposed to insure that the vector, *Aceria tulipae* Keifer, has perished. A delay of 1 week after a thorough cultivation is considered sufficient. In addition to the possible lack of moisture and nitrogen to which this spring wheat crop may be subject, it may also suffer from factors associated with the decomposition of the winter wheat seedlings. In greenhouse tests, emergence and seedling growth of wheat were significantly reduced when it was sown in non-sterile soil to which wheat seedling leaf material had been added. Neither sterilized nor non-sterilized wheat leaves produced this growth-inhibiting effect in sterile soil. Wheat seedlings appear more capable than mature plants of producing this growth-inhibiting effect in the soil.

Penicillium clove rot of garlic. SMALLEY, EUGENE B. During the 1953–1954 season many garlic plantings in California were heavily affected with a *Penicillium* clove rot. Field examinations in December, 1953, showed that from 90–95 per cent of the planted cloves were infected to some degree. Later field observations in January, 1954, indicated that about 5 per cent of the young developing plants were severely damaged from the effects of this infection. These effects showed up either as dead plants, or as wilted, yellowed, and stunted plants. The fungus isolated from the diseased garlic plants was tentatively identified as a strain of *Penicillium digitatum* Sacc. This isolate from garlic was compared with several other *Penicillium* spp. isolates from other hosts by means of single spore inoculations on mechanically injured garlic cloves. The only isolate having the ability to produce typical rotting was the *Penicillium digitatum* Sacc. from garlic.

Branch wilt of the walnut tree in relation to sunburn damage. SOMMER, NOEL F. Branch wilt, a destructive disease of Persian walnuts, has been shown to be intimately associated with sunburn damage. Naturally infected branches almost invariably show signs of prior sunburning. The pathogen, therefore, intervenes to convert a comparatively minor damage into severe injury and death to fruiting branches. The incitant, *Hendersonula toruloidea* Nattrass, is a wound parasite but produces only a low percentage of

cankers when inoculated into non-sunburned limbs. Most infections are promptly walled-off by wound periderm activity. Test branches were exposed to sunburning in July by bending as though weighted down by a heavy crop. As soon as sunburning was apparent, inoculations were made by breaking the periderm and dusting dry arthrospores over the surface. Within 2 months, over 90 per cent of the inoculated branches developed cankers extending for considerable distances into non-sunburned portions of the branches. Noninoculated branches were only slightly affected by the sunburning. The freshly sunburned tissue apparently aids disease development in 2 ways; it produces a watery exudate providing ample moisture for spore germination, and provides a medium on which the fungus can establish itself and gain access to surrounding healthy tissue.

Effect of heat treatment tests on the germination and yields of sugarcane. STEIB, R. J., M. M. THAUNG, AND LO WANG. The ratoon stunting disease of sugarcane produces no definite exterior leaf or stalk symptoms. Evidence indicates the causal agent to be a virus. Heat treatment of stalks before planting inactivates the virus but often causes serious reduction in germination. Hot water treatment of cane stalks at 50° C. for 2 hours, 51° C. for 1½ hours, 51° C. for 2 hours, 52° C. for 1 hour and 52° C. for 1½ hours were used to treat 15 varieties of cane, and germination was checked in the greenhouse. Hot air at 54° C. for 8 hours was also used. Germination of the cane was seriously lowered with all hot water treatments in 1 or more tests. The hot air treatment was safe in all tests, resulting in a slight reduction in germination. Yield tests from treated cane showed an increase in the plant cane crop and much more vigorous growth in the first stubble crop with susceptible varieties in comparison with untreated cane.

Acceleration of spore germination in Cladosporium cucumerinum. STODDARD, E. M. An assay has been developed for evaluating cucumber varieties and fungicidal treatments in control of *Cladosporium cucumerinum*. In these studies spores of *Cladosporium* lost ability to germinate when they had been isolated in culture and transferred twice. This resulted from lack of a growth factor, as shown by a renewed germinability when cucumber juice was added to spore suspensions. This factor was heat stable, not ether extractable and not precipitated by alcohol. A casamino acids nutrient also contained the required factor for germination. Glucose, mineral components, and trace elements did not affect germination, but crude casein hydrolysate caused moderate germination and yeast extract caused pronounced germination. The growth factor requirement of *Cladosporium cucumerinum* may be useful in scab control by use of competitive inhibitors or by offering plant breeders a criterion for resistance.

Chemotherapeutic control of cucumber scab. STODDARD, E. M. Soil applications of organic chemicals have given significant control of cucumber scab caused by *Cladosporium cucumerinum* in greenhouse tests. One-week-old cucumber plants growing in sand received 10 successive applications of the chemicals being tested. After the last application, plants were inoculated by spraying with a spore suspension of the causal organism in a water extract of cucumber leaf tissue and incubated for 6 days in a moist chamber at 20° C. Degree of infection was read on a scale of 0 to 4. Twenty-two materials were tested of which 4 gave significant control. These 4 are given with appropriate concentration and index of disease: maleic hydrazide, 250 p.p.m., 0.15; Orthocide 406 (captan) 2500 p.p.m., 0.35; crude Terramycin, 5000 p.p.m., 0.35; 9-cyanoethyl-carbazole, 125 p.p.m., 0.45. With a series of 9 growth promoting substances, only maleic hydrazide gave significant control. Both soil and foliage applications of Orthocide 406 were effective. In contrast to crude Terramycin, the pure material gave no control. The mode of action of effective materials has not been determined, but spore germination tests in water extracts of the treated plants indicate that spore germination may be inhibited.

Rhizoctonia aerial blight, a threat to Lespedeza production in Louisiana. STROUBE, WILLIAM H. The acreage of Lespedeza harvested for seed in Louisiana declined from 10,488 in 1945 to less than 1000 in 1952. Diseases have been established as 1 of the major factors contributing to this decline. Rhizoctonia aerial blight was found in each of 208 Lespedeza fields visited during 1951, 1952, and 1953, and was considered the most serious disease present. Isolations from Lespedeza and 40 other species and varieties of plants throughout Louisiana indicate that the disease is widespread. In cross-pathogenicity studies, isolates from a number of hosts were shown to be highly pathogenic on Lespedeza. The prevalence and destructiveness of this disease in Lespedeza seed fields led to the conclusion that it was the major cause of the decrease in Lespedeza seed production in Louisiana, which has rsulted in a shortage of seed of the adapted varieties in the state. Various attempts to find a practical control for this disease have resulted in failure.

Histology of fungus mat development in wilting oak trees. STRUCKMEYER, B. ESTHER, AND J. E. KUNTZ. Extensive plugging of the xylem vessels with tyloses and gums preceded foliage wilt in northern pin oaks infected with *Endoconidiophora fagacearum* Bretz. Mycelium was observed rarely in the vessels before or at the time of incipient wilt. Later, during severe wilt and defoliation, extensive mycelium developed in the xylem vessels. Mycelium emerged from the vessels by penetrating the pits to the ray parenchyma and xylem parenchyma cells. Mycelium continued to progress radially through the pits of the ray cells to the cambium. Mycelial penetration was usually intracellular, but occasional intercellular penetration was observed. The ray cells frequently proliferated; those near the cambium commonly collapsed and disintegrated. Mycelium formed mats between the wood and bark. During mat formation, wood and bark separated and the bark soon cracked. Mycelium penetrated some bark tissue. In most mats, a central core of hyphae was surrounded by a pseudo-parenchymatous stroma of variously enlarged, rounded, fungus cells. Within the stroma were distinct, compact structures consisting of 2 or more large cells surrounded by several layers of smaller cells.

Transmission of lettuce mosaic virus by the green peach aphid. SYLVESTER, EDWARD S. Transmission of lettuce mosaic virus by the green peach aphid, *Myzus persicae* (Sulzer), to lettuce seedlings (variety Great Lakes) indicated that the acquisition threshold period is 10–15 seconds, with an increase in efficiency with feedings up to at least 30 seconds. The inoculation threshold period is in the 5–10 second range, with no benefit resulting from lengthening a single test plant penetration to 30 seconds. Pre-acquisition feeding fasting periods as short as 5 minutes can increase the probability of obtaining infective insects. Decrease in vector efficiency occurs when infective aphids feed for prolonged periods on either a virus source plant or a virus-free plant. Virus loss also occurs if infective aphids are fasted. The most rapid loss of efficiency occurs when the insects are fed on a virus-free plant following acquisition of a virus charge. Less rapid loss of charge occurs if infective aphids are allowed to remain for prolonged periods of time on a virus source plant, whereas maximum retention of virus occurs if infective insects are fasted before being fed on a test plant.

Nematicidal trends in some hydroxybenzenes. TARJAN, A. C. Evaluation of mono-, di-, and tri-hydroxybenzenes as contact, ovicidal, and therapeutic nematicides has indicated that the type of substituted radical and its position on the benzene ring may affect the efficacy of the compound. *Panagrellus redivivus* was used in contact tests, whereas egg masses of *Meloidogyne incognita* were employed in ovicide tests. Therapeutic tests were conducted against *M. incognita*-infected tomato plants. Both m- and p-hydroxybenzoic acid were found to be better ovicides than o-hydroxybenzoic

acid. Catechol proved to be an ovicide superior to either resorcinol or hydroquinone. Among resorcinols with a radical substituted in the 4 position, hexyl resorcinol was found to be an excellent contact nematicide and a good ovicide, whereas 2,4-dihydroxy benzaldehyde displayed some effectiveness as an ovicide. Monochlororesorcinol, 2,4-dihydroxy acetophenone, 2,4-dihydroxy benzoic acid, and 4-propionyl resorcinol showed no nematicidal activity. Resorcinols with methyl and pentadecyl groups substituted in the 5 position were ineffective as nematicides as were the diacetate, the diethyl ether, and the dioctyl ether of 5-pentadecyl resorcinol. Contact activity of 2,4,6-trinitro resorcinol was very good indicating the potential nematoxicity of substituted nitro groups. Resorcinol diacetic acid and resorcinol diacetate were fair contact nematicides, whereas resorcinol dibenzoate was ineffective. Position of hydroxy groups and substitutions in the trihydroxybenzenes seemed to have little effect in enhancing nematicidal action.

Effect of brief exposures at 40° C. on germination of sporangia of Phytophthora infestans. TAYLOR, CARLTON F., JOHN J. SMOOT, D. O. QUINN, R. A. ROHDE, AND EDWARD S. ELLIOTT. Exposure of sporangia of *P. infestans* at a temperature of 40° C. markedly altered the rate at which direct germination occurred. Germination of sporangia was observed on peanut hull agar incubated at 20°C. Unheated sporangia germinated at a linear rate reaching the maximum in 30–48 hours. Treatment at 40°C. induced a lag period followed by rapid germination which approached the maximum value in a short period of time. The duration of lag period and total germination varied with length of exposure. The final value for germination of sporangia exposed for 2½–10 minutes exceeded that of controls. With longer treatment there was a progressive reduction in viability. In water at 10°C. indirect germination, i.e., escape of the contents as zoospores, of the untreated sporangia was rapid (22 per cent at 1 and 70 per cent at 6 hours). Exposure at 40°C. for 1, 2½, and 5 minutes delayed the start of indirect germination to 3, 6, and 8 hours respectively with the final germination at 48 hours being 46, 42, and 18 per cent. Some of the remaining sporangia eventually produced germ tubes with 39 per cent having developed by 48 hours following the 5 minute treatment.

Factors affecting development of necrosis in some bean varieties inoculated with common bean mosaic virus. THOMAS, H. REX. Bean varieties, such as Topcrop or Contender, with resistance to common bean mosaic virus controlled by a dominant factor derived from Corbett Refugee bean, can, under certain conditions, develop necrosis when inoculated with common bean mosaic virus or the strain of this virus infectious to Michelite or Great Northern U. I. No. 15 bean. When young bean plants were exposed continuously to temperatures of 32°C. or above, veinal necrosis was observed in the inoculated leaves within 40 hours. This necrosis increased and within 4 or 5 days the plants died. Growing inoculated plants in soil with temperatures of 20°, 25°, and 30°C. had little effect on the development of leaf necrosis when the air temperature was approximately 20° at night and 25° during the day. Keeping plants at approximately 16°C. for 4 days or longer immediately after inoculation greatly reduced the development of systemic necrosis when plants were subsequently kept at 20° or above. After inoculation, exposure for 4 hours at 32°C. and then at approximately 20° for the remaining 20 hours during each day was sufficient to induce leaf veinal necrosis within 3 days.

Studies on the mechanism of fungicidal action of the ethylenebisdithiocarbamates. 2. Theoretical considerations. THORN, G. D., AND R. A. LUDWIG. The ethylenebisdithiocarbamates are converted to ethylenethiuram monosulfide (E.T.M.) on the plant. The latter compound is probably not the fungicide itself, but acts through rearrangement to an isothiocyanate which is the toxic principle. The increased fungicidal activity of E.T.M. in the presence of metal salts and the demonstration of isothiocyanate formation support this postulate.

Genetic segregation and heterocaryosis in Helminthosporium sativum. TINLINE, R. D., AND J. G. DICKSON. The inheritance of several characters in *Helminthosporium sativum* was studied in different hybrid populations. Segregation ratios could not be determined accurately for pathogenicity, growth rate, and conidial production since segregation was largely indistinguishable from normal variation. Some isolates derived from randomly selected ascospores differed significantly in pathogenicity from one another and from the common parental isolates. The distribution curves of data indicated recombination and segregation of the factors. The data obtained when the 4 pairs of isolates derived from the 8 ascospores of an ascus were compared with the parental isolates for differences in pathogenicity suggested that multiple factors controlled pathogenicity. Conidial color and pathogenicity appeared to be inherited independently. Conidial colors were employed as markers to indicate heterocaryosis achieved through hyphal anastomosis. Anastomoses occurred rarely between the hyphae of morphologically distinct isolates but frequently between the hyphae of a single isolate. In anastomosing cultures, all the conidia borne on a conidiophore were alike in color and isolates derived from conidiophore progeny appeared identical. The pre- and post-anastomosis hyphae produced conidia similar in color to the spore from which the hyphae originated. Apparently, therefore, some mechanism inhibited or masked the establishment or expression of heterocaryosis.

Damage caused by Polyporus hispidus on bottomland red oaks. TOOLE, E. RICHARD. *Polyporus hispidus* is frequently found in the South associated with cankers and heart rot on the main stems of the bottomland red oaks. Investigation in 1 area showed that 13 per cent of all the willow oaks and 3 per cent of the Nuttall oaks in the stand were affected. Most cankers were within 16 ft. of the ground. Seven per cent of the willow oak cubic ft. volume was cull due to *P. hispidus*. Analysis of 55 cankers revealed that the heart rot exceeded the length of the cankers by 2.38 ± 0.993 ft., and that the length of cankers and rot increased $0.484 \pm .017$ ft. per year.

The etiology, development, and control of tomato fruit tumor. TRESHOW, MICHAEL. Tomato fruit tumor, previously referred to as oedema or waxy-blister, is characterized by slightly raised, irregularly shaped, succulent tumors usually a few mm. in diameter but occasionally involving half the fruit surface. The development of intumescences is probably stimulated by traumatin which forms when immature fruit is rubbed. Cells of the outermost pericarp parenchyma enlarge and divide within 24 hours of rubbing, crushing the subepidermal collenchyma and stretching the epidermis. Minimum and maximum temperatures for tumor development are 21° and 33°C. Rate of tumor formation is directly proportional to temperature within this range. Tumors usually occur on 10–20 per cent of the mature green fruit in the field and increase proportionally in extent and frequency to the amount of handling during packaging operations. Fruits handled in the pink and ripe stage are not susceptible to tumor formation. Tomato fruit tumor may be controlled by careful handling to eliminate scuffing of fruit, processing pink or ripe fruit rather than mature greens, or by ripening the fruit at less than 18°C.

The control of loose smut in barley. TYNER, L. E. Newal barley carrying 10 per cent natural infection with loose smut (*Ustilago nuda*) was soaked in water at 72°F. for 64–90 hours. The smut was completely eliminated and there was no significant difference between the number of mature plants in the treated and control plots. It was evident that elimination of the smut mycelium did not impair ability of the infected embryos to produce normal healthy plants. Complete control of loose smut was effected by soaking the seed at 72–77°F. for 56 hours in water in sealed containers in which the carbon dioxide or the oxygen was absorbed by chemicals. Further, when these gases were bubbled through tubes of water in which seed was soaked

there was no apparent difference in smut control between these treatments and the water-soak control.

Screening fungicides non-toxic to germinating tree seed. VAARTAJA, OLLI. A petri dish method was developed for testing rapidly fungicidal and chemotherapeutic chemicals against pathogenic fungi and their toxicity to germinating seed. Virulent strains of *Rhizoctonia solani* and *Pythium* were used as pathogens, and *Pinus banksiana* and occasionally *Betula verrucosa*, as hosts. Most of 65 chemicals tested were either ineffective against the fungi or toxic to seeds at effective concentrations. Certain chemicals allowed maximum germination and development of seedlings despite the presence of the pathogen. Intermediate results were rare. Chemicals effective against *Rhizoctonia*, were also effective against *Pythium*. Generally both tree species gave similar results. Nabam, zineb, ferbam, Manzate, Vancide-51, Spergon (Chloronil), lindane, heptachlor, boric acid, benzoic acid, oxyquinolene, malachite green, lauryl sulfate, and Thiolutin, performed well at certain concentrations. Thiram, captan, and especially Rimocidin were outstanding, giving good protection at widely varying concentrations. Actidione, Gladiolic acid, and Helixin gave fair or good results with *Betula*, but were mostly toxic to *Pinus*. Gladiolic acid, Streptomycin, and Bordeaux mixture were quite effective against *Pythium*, but less effective against *Rhizoctonia*. Trinitrotoluene gave good control; however, this was partly indirect, due to the peculiarly encouraged growth of antagonistic *Trichoderma* contaminants.

Tobacco and zinnia—two new herbaceous hosts for Prunus virus. VARNEY, E. H., AND J. DUAIN MOORE. In 1952, virus was transmitted by mechanical inoculation from leaves of certain *Prunus* species to tobacco and zinnia. Symptoms on Havana 38 and Kentucky 56 tobacco consisted of concentric rings and line patterns and were more pronounced on Kentucky 56 than on Havana 38. On zinnia, leaf symptoms ranged from a faint, temporary mottle-mosaic to a striking, permanent yellow and white mottle. Zinnia inflorescences showed some flower breaking. Virus transmissions to tobacco and zinnia have been obtained from sour cherry, mahaleb cherry, peach, and Italian Prune. Transmissions were made to cucumber from all trees from which transmissions to tobacco and zinnia were obtained, but not all sources that transmitted to cucumber would also serve as a source of inoculum for tobacco and zinnia. Likewise, it was not possible to transmit to zinnia from all sources that would infect tobacco. It is not known whether these differences are due to separate viruses or to different strains of the same virus.

Altering disease resistance with ionizing radiation. WAGGONER, PAUL E. Exposure of tomato plants to soft X-rays can increase or decrease resistance to Fusarium wilt. Exposure of the shoot before or at inoculation time does not alter resistance significantly. Exposure of the whole plant to 5–10 Kr at inoculation time has little effect but a dose of 20 Kr causes resistance to be decreased. In contrast, if the roots alone are exposed to 20 Kr, resistance is increased. When plants are exposed to radiation 5–10 days before inoculation, they respond to lower doses and in a different manner. Exposure of the roots or whole plants to more than 5 Kr increases resistance. Three weeks later such plants are larger than inoculated checks. Failure of shoot irradiation or half-root irradiation to alter resistance indicates that alteration is not achieved through changed auxin level or toxin production. The consistency of the alteration suggests it is not genetic. (Atomic Energy Commission support is acknowledged.)

Pectic enzymes produced by Fusarium oxysporum f. lycopersici in infected plants. WAGGONER, PAUL E., AND A. E. DIMOND. Samples of vascular sap exuded from healthy and from Fusarium wilted tomato plants were mixed with 2 volumes of pectin solution containing sodium chloride and methyl red at pH 5. The pH, measured colorimetrically, dropped more rapidly in the solution containing the diseased than in that containing healthy plant sap. The drop in pH in the solution containing diseased plant sap was not retarded by a wetting agent, a characteristic of pectin methylesterase from *Fusarium* but not of that from tomato. Therefore, pectin methylesterase is produced in infected plants by *Fusarium*. After 48 hours under toluene more reducing sugars were present in the pectin solution containing diseased than in the solution containing healthy plant sap. Therefore, since tomato stems do not produce polygalacturonase, that enzyme is produced by *Fusarium* in infected plants. Because substrate is present and inhibitors are absent in the vascular stream where the pH is near the optimum for both fungal enzymes, it is highly probable that pectin hydrolysis will occur in the xylem. Therefore, hypotheses concerning wilt production should take into consideration the maceration of cells in the xylem and the appearance of the hydrolytic products in the stream for at least a short time.

The Fusarium phase of the root-disease complex in coffee. WELLMAN, FREDERICK L. Artificial inoculations of *Coffea arabica* seedlings with isolates of *Rosellinia bunodes, R. pepo, Fomes* spp., and *Gloeosporium* obtained from diseased coffee roots have given negative results. A *Corticium* species and *Pellicularia filamentosa* caused only soreskin and damping-off. *R. bunodes* and a *Fomes* caused infection when host plants were grown in warm, unsterilized nursery soil. Other fungi less frequently isolated from diseased seedlings included 2 *Fusaria* and *Gloeosporium cingulata*. Occasionally certain bacteria were obtained as well as species of *Trichoderma, Pestalozzia, Cytospora*, and *Diplodia*. A strain of *Fusarium roseum* was found occasionally to be mildly parasitic. The most important vascular invader was identified as *Fusarium oxysporum f. coffeae* n. comb. Various strains caused a mild to severe attack of seedling roots. *Gloeosporium* and *Rosellinia* spp., when isolated, were found mostly accompanied by *F. oxysporum* f. *coffeae*. *Fusarium* infected seedlings may readily recover. Detailed search demonstrated this *Fusarium* of primary occurrence where overwhelming appearance suggested other root decays fungi as sole causative factors. *Coffea dewevrei* is apparently resistant to *Fusarium*. Control of root rots are being sought by use of dewevrei roots grafted with arabica tops.

Fungi isolated most frequently from dying coffee fruit branches. WELLMAN, FREDERICK L. A serious condition called dieback results from unseasonable leaf fall of coffee. In Costa Rica during the last 4 years more than 2000 isolations have been made. The following genera of fungi were isolated from lateral branch tissues: *Gloeosporium, Pestalozzia, Pellicularia, Fusarium, Penicillium, Cercospora, Diplodia, Aspergillus, Trichoderma*, 2 sterile white fungi, and 1 fungus with black stroma. Inoculation tests gave erratic results. Isolates of *Gloeosporium, Fusarium, Penicillium*, and the fungus with black stroma appeared to be mildly pathogenic. Isolates of *Gloeosporium, Fusarium, Pestalozzia*, and *Diplodia* were obtained from stem leaf traces below places left bare after premature leaf drop. Fungi were also isolated from internal tissues of debilitated leaves just before drop, also from petioles, mid-veins and mesophyll. The most vigorous trees showed least leaf fall. Less disease occurred in trees in moderate shade than in full sun and in well-weeded plantings than in severely grass-infested plots. Sprays of inorganic and organic fungicides increased leaf retention and greatly reduced dieback of lateral branches.

Cottony blight of ryegrass caused by Pythium aphanidermatum. WELLS, HOMER D., AND B. P. ROBINSON. On October 18, 1953, ryegrass turf plots at Tifton, Georgia, were affected with an aerial blight caused by *Pythium aphanidermatum*. The disease first appeared as small, irregular white spots in the turf. These spots later became very pronounced and took on a definite cottony appearance. The latter resulted from the profuse growth of light-colored aerial hyphae and appeared sufficiently characteristic of the disease to warrant the use of the proposed common name "cottony blight." Infected blades at first appeared soft and

greasy and matted together. The affected plants rapidly turned brown, all susceptible plants being killed. The disease was associated with warm weather and was halted with the onset of cool weather. The disease has been observed only on seedlings. Control of the disease by spraying with Calocure, Cadminate (cadmium succinate), Actidione, PMAS, Mercadamine, tersan, tersan plus PMAS, tersan plus Cadminate, and tersan plus Mercadamine, Dithane (zineb) plus salicylic acid, and Bordeaux mixture was unsatisfactory. However, daily applications of PMAS, tersan plus PMAS, Dithane plus salicylic acid, and Actidione showed some promise of control. Treating topdressing material with one lb. of methyl bromide per 10 cu. ft. of soil was effective in preventing the introduction of the pathogen in the topdressing material.

Two additional mating reaction genes in Glomerella. WHEELER, H. E. Previous studies have shown that mating reactions in *Glomerella cingulata* are controlled by genes at several loci. At each of 2 of these loci, designated A and B, 3 alleles are known. One of the new mating reaction genes represents a fourth allele at the B locus. In addition to its effect upon mating reactions, this gene renders normally self-fertile cultures completely self-sterile. The second gene, although not definitely located, apparently involves a new locus. Although this gene has no effect on self-fertility, it causes cross-sterility in matings involving cultures which carry the wild-type allele at the B locus. This occurs despite the fact that such matings produce strong positive reactions, evidenced by the rapid formation of dense ridges of perithecia between the mated cultures, both of which may be self-fertile.

Verticillium wilt resistance in strawberry. WILHELM, STEPHEN. Dipping washed roots of strawberry seedlings in a virulent spore suspension of *Verticillium* or spraying exposed roots with spores produces infection in nearly all susceptible individuals in 8–10 weeks. Fluctuating temperatures and light intensity as well as high nitrogen level in the soil favor symptom expression. Resistance and susceptibility grade with each other in strawberry. Immune individuals as defined here develop no symptoms and grow out from induced infection, or cannot be infected. Four crosses (susceptible × susceptible) produced 0 per cent of immune plants; 4 crosses (susceptible × resistant) produced 4–20 per cent of immune plants, and 5 crosses (resistant × resistant) produced 21–48 per cent of immune plants. Studies were made of 1800 seedlings. California selections of *Fragaria chiloensis* were immune; *F. virginiana* and *F. vesca* were susceptible. Verticillium immune plants generally have thick, glossy dark green leaves suggestive of derivation from *F. chiloensis*, and 95 per cent are also resistant to powdery mildew of the foliage. Verticillium wilt resistance is not linked to reaction to virus yellows, nor to any particular fruit characteristic.

Two new fungus root parasites of strawberry. WILHELM, STEPHEN, AND PAUL E. NELSON. Two fungi, apparently heretofore unknown, have been isolated repeatedly from diseased strawberry roots and their pathogenicity to strawberry established in controlled greenhouse inoculations. These fungi are thought to account for much of the strawberry root deterioration in soils where the black root disease occurs. One is a species of *Pyrenochaeta*, often sterile, sporulating in culture under special conditions with minute conidia in setose pycnidia and producing microsclerotia, which typically fill the lumina of cortical root cells. It differs in microsclerotial characteristics from *P. terrestris* and lacks pink pigment. The other fungus produces non-septate, hyaline lunate conidia in dry heads on short distinct conidiophores of the Ramularia type. Characteristic chlamydospore masses are produced in culture and in the outer cortical tissues of infected roots. These primary parasites may pave the way for invasion by saprophytes from the rhizosphere which may become definitely pathogenic after entrance.

New indicator hosts for the little cherry disease. WILKS, J. M., AND M. F. WELSH. Symptoms of the Kootenay little cherry disease are normally confined to the fruit. Studies have been in progress to discover *Prunus* varieties in which foliar symptoms are also expressed. In 26 of the 54 varieties under test, a premature interveinal reddening on 1 or both surfaces of the leaf laminae was noted. This symptom first appeared about July 1, at which time there was no red coloration apparent on the leaves of the check trees. Maximum development of color occurred after periods of cool weather. Ringspot viruses and other viruses not in the little cherry group, such as rusty mottle, mora, and cherry yellows failed to produce these symptoms. Western X little cherry virus at The Dalles, Oregon, did produce foliar symptoms similar to those caused by Kootenay little cherry.

Seed-transmission tests of almond bud failure disorders. WILSON, E. E. A bud failure disorder of Nonpareil and Peerless almond varieties is scion-perpetuated but not graft-transmitted. A bud failure disorder of the Drake variety is both scion-perpetuated and graft-transmitted. Recent tests indicate that the 2 disorders also differ with respect to seed-transmission. After 4 years, none of the 100 seeds taken from affected Drake trees produced seedlings with symptoms, whereas 21 of the 300 seedlings produced from seed taken from affected Nonpareil and Peerless trees manifested symptoms. Moreover, certain Nonpareil seedlings, which did not exhibit symptoms after 4 years, were shown to carry the disorder when propagated on an appropriate rootstock. The Jordanolo (Nonpareil × Harriott), a variety in which the Nonpareil-Peerless type of bud failure is extremely prevalent, probably acquired the disorder through seed from the Nonpareil parent. Other hybrid Nonpareil seedlings developed in a breeding program at this station manifested a similar disorder or were found to carry the disorder when propagated for testing.

Sugar utilization by some species of Endoconidiophora. WILSON, E. M. Six species of *Endoconidiophora* transformed sucrose into oligosaccharides when grown on a sucrose-asparagine medium. *E. coerulescens*, *E. moniliformis*, *E. virescens*, and an albino mutant of *E. fagacearum* produced oligosaccharides when grown on a maltose-asparagine medium. Oligosaccharides formed by these species and under the conditions used in this study were found during the early growth phase. After 5 days of incubation on a sucrose medium, *E. virescens* transformed sucrose into 2 distinctly different oligosaccharides and into glucose and fructose. *E. virescens*, *E. fagacearum* normal, and *E. fagacearum* albino mutant, when grown on a sucrose medium, produced filtrates in which fructose persisted after oligosaccharides, sucrose and glucose had disappeared. After an incubation period of 44 days, filtrates of *E. fagacearum* on maltose, raffinose, lactose, and sucrose media contained polysaccharides, which precipitated when acetone was added. Acid hydrolysis of these polysaccharides yielded glucose. After an incubation period of 18 days, filtrates of *E. fagacearum* on a glucose medium contained only a slight amount of material precipitable by acetone as compared to filtrates of the above mentioned sugars.

The interrelationship of potato and tomato races of Phytophthora infestans. WILSON, J. B. The reaction of 29 potato isolates of *P. infestans*, representing 10 different potato races, were studied on tomato differential hosts, and 10 tomato isolates, representing 3 different tomato races, were studied on potato differential hosts. The potato isolates fell into 4 groups: Group I, weakly to non-pathogenic on all tomato varieties; Group II, pathogenic on the recessive varieties Marglobe, Rutgers, and Bonny Best, but non-pathogenic on minor-gene varieties Wisconsin "55" and #19 and dominant-gene varieties #36 and #106; Group III, pathogenic to the recessive and minor-gene varieties but not to the dominant-gene accessions; Group IV, pathogenic to all tomato varieties. Isolates of Group II and III appear to differ only in degree of virulence. On potato hosts, the tomato isolates reacted as potato races 0, 3, or 4. One isolate of a given potato race may react as one tomato race, while another may react as a different tomato race.

A similar relationship exists in regard to tomato races on potato. The tomato genes are different from any now known in potato; the race characteristic of a given isolate on potato should be considered independently from its characteristic on tomato.

Streptomycin most effective in controlling fireblight of apple and pear. WINTER, H. F., AND H. C. YOUNG. Laboratory, greenhouse, and orchard tests in 1953 showed that streptomycin and terramycin inhibited growth of *Erwinia amylovora* and largely prevented fireblight infection on inoculated Jonathan apple trees. Streptomycin was most effective in the orchard. When applied at 120 p.p.m. at early bloom, full bloom and petal fall, it allowed only 2 per cent blossom cluster infection and 0 per cent twig infection. Untreated but inoculated trees showed 83 and 20 per cent blossom and twig infection, respectively. Three applications of various streptomycin formulations at 100 p.p.m. in numerous tests on apple and pear trees, both inoculated and noninoculated, gave excellent control of fireblight in 1954. Terramycin and tetracycline were less effective than any streptomycin formulation. Early and full bloom applications were most important. On inoculated 4-year-old Bartlett pear trees, 3 applications of 100 p.p.m. streptomycin allowed 0.37 per cent twig infection as compared to 60.2 per cent on controls. On noninoculated 6-year-old Bartlett in a commercial orchard, Agrimycin at a dosage to give 100 p.p.m. streptomycin allowed 0.42 infections per tree as compared with 9.5 per untreated tree. Streptomycin at 50 p.p.m. on noninoculated apple trees gave excellent commercial control of fireblight but on inoculated trees was inferior to the 100 p.p.m. dosage.

Helminthosporium sativum becomes destructive on hitherto resistant varieties of barley. WOOD, L. S., J. J. CHRISTENSEN, AND J. W. LAMBERT. *Helminthosporium sativum* is one of the most destructive pathogens of barley in the north central states; sometimes it causes enormous loss to barley. Helminthosporium blight, although prevalent for some years on Kindred in pathological gardens, did not become generally destructive on that variety until 1953. In that year losses amounting to 50 per cent of the potential crop occurred in certain areas. All commercial varieties, like Kindred, Mars, and Barbless, originally resistant to *H. sativum*, are now susceptible to certain races prevalent in Minnesota. *H. sativum* comprises many parasitic races that differ greatly in their parasitic ability, not only on wheat, rye, oats, and corn, but also on varieties of barley. Pathogenicity tests with many isolates indicate that races now virulent on Kindred are prevalent and widely distributed. During the past 4 years numerous varieties and thousands of hybrid lines were subjected to artificial epidemics of *H. sativum* created by using a mixture of biotypes and races. Most of the material tested was susceptible. Some of the more promising materials were selections of Manchuria and Brandon and a few hybrids derived from diverse crosses.

Acquired immunity from bean rust. YARWOOD, C. E. When Pinto bean primary leaves infected in localized areas with the uredinial stage of *Uromyces phaseoli* were reinoculated, infection from the second inoculation did not occur close to the first infection. Inhibition of the second inoculation extended to a greater distance distally than proximally from the first infection. The older the first infection, the greater the distance of inhibition of the second infection. Spores exposed to emanations from infected leaves or from germinating spores showed poorer germination than similar spores exposed to healthy leaves. Detached, rusted bean leaves held close to bean, broad bean, or snapdragon leaves inoculated with their respective rusts, prevented infection with these rusts. When bean rust urediospores were seeded on agar, the percentage germination decreased as the concentration of rust spores increased, but the length of germ tubes of germinating spores was not decreased. When rust spores were killed with heat or when rust mycelium in infected leaves was killed with heat without injuring the leaves, their property of reducing spore germination at a distance was retained in part. Rusted bean leaves did not reduce infection with bean powdery mildew, bean anthracnose, tobacco mosaic virus, or tobacco necrosis virus.

Mechanical transmission of 3 fruit tree viruses. YARWOOD, C. E., AND H. E. THOMAS. By means of quick tissue inoculation and K_2HPO_4 or Na_2SO_3 with the inoculum, an apple mosaic virus, a virus from rough bark of plum, and a virus from peach yellow bud mosaic have been transmitted mechanically. Apple mosaic virus has been transmitted from apple to bean, tobacco, cucumber, tomato, cowpea, and broad bean by means of tissue, and from tobacco to apple, tobacco, and broad bean by means of *Cuscuta subinclusa*. Mechanical inoculations from apple to apple or from tobacco to apple were unsuccessful. Tobacco infected with apple mosaic virus first showed local lesions, then a severe shock effect, and then recovery with acquired immunity. Mechanical inoculations with a virus from plum rough bark have been successful only on cucumber, where chlorotic lesions, systemic mosaic, and death resulted. Mechanical inoculations with a virus from peach yellow bud mosaic yielded local lesions and systemic infection on several bean varieties and on cowpea. After the viruses from apple mosaic, plum rough bark, and peach yellow bud mosaic were established on tobacco, cucumber, and bean respectively, by means of pinch tissue inoculations, they were readily transmitted to these latter hosts by means of ordinary juice inoculations. By the same methods, mechanical inoculations with 15 other virus diseases of woody plants were unsuccessful.

A study of soil aeration and other factors in relation to the littleleaf disease of pine. ZAK, B. Shortleaf and loblolly pine seedlings were grown in the greenhouse for 2 years under various degrees of soil aeration, soil wetness, soil texture, and with and without *Phytophthora cinnamomi* in the soil. Low O_2 (5 per cent) in the soil atmosphere caused injury to both species but loblolly appeared less affected than shortleaf pine. High CO_2 (9 per cent) with adequate O_2 did not reduce growth of either species. Inoculation tests using *P. cinnamomi* showed that shortleaf roots are much more susceptible to attack than are those of loblolly pine. Poor soil aeration did not appear to affect pathogenicity of this fungus except possibly indirectly by predisposing the roots to attack. Injury was greatest in heavier clay soils. A combination of heavy watering and attack by the fungus caused considerable damage to the roots of shortleaf pine in these soils. The relatively low susceptibility to root injury by this water mold and the greater tolerance to poor soil aeration of loblolly pine, explains to a large degree the much smaller incidence of littleleaf in this species than in shortleaf pine.

Chemotherapy for control of Phytophthora root rot of avocado. ZENTMYER, GEORGE A. Systemic fungicides have some effect when applied to soil, stems, or leaves for control of vascular or foliage diseases. Five chemicals reported to have systemic properties (2,4,6-trichlorophenoxyacetic acid, pentachlorophenoxyacetic acid, sodium 2-benzothiazyl thioglycolate, 8-hydroxyquinoline benzoate, Bayer 19117) were tested for translocation to the roots and control of Phytophthora root rot by applying as foliage sprays and injecting stems of avocado seedlings. Plants were sprayed before transplanting into infested soil, and at intervals thereafter, with 0.1 per cent solutions or suspensions. Tops of other plants were severed and a reservoir of chemical attached at the time of transplanting into infested soil. Data were taken on progress of root infection, wilting, development of new roots; injected plants were dissected, and sections cut at 1 in. intervals from point of injection to lower tap root were used in a biological assay on agar to determine distribution of chemical. Spraying with 8-hydroxyquinoline benzoate markedly retarded progress of wilting and increased new root development over that of controls; retardation of invasion of roots was slight. Neutralization of a fungus toxin within the plant is indicated. Other materials showed little or no effect. Biological assay, using *Phytophthora cinnamomi*, indicated accumulation of a

fungicidal material in the upper tap root in plants injected with 8-hydroxyquinoline benzoate and 2,4,6-T.

A bimodal fungitoxicity curve for zineb. ZENTMYER, GEORGE A., AND J. L. ERSPAMER. Bimodal curves for the fungitoxicity of zineb (zinc ethylene bisdithiocarbamate) to *Phytophthora cinnamomi* have appeared in tests in soil and in potato dextrose agar. Inversion of toxicity occurred when the fungicide was applied to soil in shell vials, either in suspension or mixed dry with the soil. Parzate suspensions containing over 2,000 p.p.m. zineb were not fungicidal when 5 ml. of this suspension was applied to 20 gm. of soil in vials, 1,000 p.p.m. was fungicidal, lower dosages were not fungicidal. In several tests, when Dithane Z-78 was mixed with soil, high dosages were not fungicidal, lower dosages were highly fungicidal, and still lower dosages, ineffective. A pattern analagous to Dimond et al's TMTD curve was obtained when zineb was incorporated into potato dextrose agar, with inhibition at high dosages (10,000 p.p.m. zineb based on weight of agar), a trough in the curve at 7,500 and 5,000 p.p.m., a second peak at 2,500 p.p.m., and increasing growth with decreasing dosage from 1,250 p.p.m. Possible explanations include: differential toxicity of molecular zineb and of ions or metal complexes formed, permeation effects in soil, periodic effects of colloids in agar and soil, or combinations of several factors. Results have not always followed the same pattern, indicating the influence of undetermined factors.

LISTING OF BOOKS

PHYTOPATHOLOGY will, from time to time as space becomes available, list titles of books on phytopathological subjects. A brief description of the contents of the books will be included.

DE ONG, E. R. 1953. **Insect, fungus and weed control.** Chemical Publishing Co. Inc., New York, N. Y. 400 p. Price $10.00.

Descriptions are given of the various pesticides and their recommendations for use. There are 17 chapters, with references, on the different groups of pesticides and fields of application. A chapter apiece is devoted to Inorganic Fungicides, Organic Fungicides, Seed Disinfectants and Protecting Agents, and Industrial Insecticides and Fungicides which includes brief information on the more common experimental materials.

KÖHLER, E., AND KLINKOWSKI, M. 1954. **Viruskrankheiten.** Ed. by O. Appel and H. Richter. Published as volume 2, part 1, of SORAUER, P., **Handbuch der Pflanzenkrankheiten.** Ed. 6, Paul Parey, Berlin. 784 p. 326 figures in the text. DM 150.

Köhler and Klinkowski's new book presents detailed descriptions of substantially all known plant diseases caused by viruses, arranged according to the taxonomy of host plants. It is exceptionally well illustrated and includes a 64-page index. Each description of a disease is accompanied by its own bibliography, with citations given in full. A comprehensive statement of the general features of viral diseases in plants and a description of techniques used in studying viruses occupy the first 132 pages.

ZUNDEL, G. L. 1953. **The Ustilaginales of the World.** Pa. Agr. Exp. Station, Room 107 Agriculture Building, State College, Pa. XII + 410 pp. 8½ × 11 in. Multilithed, bound. $4.00 postpaid. Only 550 copies printed.

An important monograph representing the life work of the late Dr. George Zundel. It consists of complete descriptions of the fungi, synonyms, type hosts, host symptoms and distribution of all the known smut species of the world, together with a host index and an index of synonyms.

THE MECHANISM OF WILTING IN TOBACCO PLANTS AFFECTED BY BLACK SHANK [1]

Harry R. Powers, Jr. [2]

SUMMARY

Black shank of tobacco is characterized by sudden, drastic wilting following the development of localized lesions on roots and the base of the stem. That this wilting was due to a localized obstruction of water movement in the area of the lesion was shown by: 1) diseased plants, even after prolonged wilting, were found to regain turgor when water was introduced into the stems above the lesions; 2) bridge grafts around stem lesions delayed wilting until the advancing lesions had reached the grafted areas; 3) aqueous solutions of eosin dye moved up to, but not through stem lesions.

Tyloses and gums, as well as abundant masses of mycelium, were found in the vessels of diseased tissue. Most of the vessels were obstructed at one level or another in the area of the lesions. Numerous hyphae in the vascular ray cells obviously retarded the lateral movement of water. This condition of the xylem tissues offered a reasonable explanation for lack of water movement through the lesions.

Tyloses were formed below cut surfaces of sections of tobacco stems immersed in water with the upper ends exposed to air. Tyloses also formed in diseased plants which had been prevented from wilting by being held in a moist chamber. Under the latter condition it can be assumed that the water columns in the vessels were intact and air was not present within the vessels. Thus tylose formation occurred with or without exposure of the vessels to air.

Severe wilting, with tylose and gum formation, developed when excised, healthy plants were placed in extracts of either healthy or diseased plant tissue. These tyloses and gums were very similar to those found in diseased tissue and gave identical reactions to microchemical tests. Except for the absence of mycelium, it was impossible to distinguish between a cross section of xylem from a stem treated with extracts and one from a black shank lesion.

It can be concluded that in black shank, tyloses and gums are a primary cause of the obstruction of water movement in diseased plants rather than a result of any previous rupture of the water columns. The evidence also indicates that tyloses and gums in the vessels of diseased stems are caused primarily by toxic effects of decomposition products of invaded cells. These toxic substances are not systemic in nature, but affect primarily a restricted region in the xylem. Moreover, they are not necessarily products of fungal metabolism since cell decomposition products alone induce a similar reaction.

According to these findings the toxin theory, as usually interpreted, does not explain the wilting in the black shank disease of tobacco. The only explanation in accord with the facts presented is that wilting is due to the blocking of water movement through the lesions.

Black shank of tobacco, incited by *Phytophthora parasitica* (Dastur) var. *nicotianae* (Breda de Haan) Tucker, is characterized by sudden, drastic wilting following development of localized lesions on the roots and the base of the stem. Severe wilting is the outstanding symptom and it is usually much more pronounced than that produced in tobacco plants infected with the vascular pathogen, *Fusarium oxysporum* (Schlect.) var. *nicotianae* J. Johnson. Once wilting occurs in black shank infected plants they do not recover and usually die within a short time. In local lesion diseases, wilting is usually considered to be a secondary symptom which follows extensive tissue invasion. The development and severity of the wilt symptom is generally related to the extent to which local lesions in roots or stems interfere with water absorptive or translocative functions of the affected plant parts. Although black shank lesions are localized, development of the wilt symptom has been attributed to systemic effects of a toxic metabolic product of the pathogen (12, 13). The possibility that wilting might be due to the disruption of water movement through the lesions has never been mentioned. However, it has been reported in early papers on black shank that all tissues of the stem and root lesions were invaded by hyphae of the pathogen (6, 10).

In view of these facts, black shank infected plants seemed to offer particularly favorable material for studying pathological wilting in tobacco. Initially the effect of black shank infection on water movement in the plant was investigated. Later, critical studies were made of the pathological histology of black shank infections in different stages of pathogensis with the view of determining the effects of the pathogen on vascular tissues. As an aid in interpreting the microscopic observations, comparative examinations also were made of tobacco plants infected by 2 other diseases: Fusarium wilt, incited by *F. oxysporum* var.

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[2] Plant Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry Station, Beltsville, Maryland. The writer is greatly indebted to Dr. C. J. Nusbaum, Professor of Plant Pathology, North Carolina State College, under whose guidance this work was done. The work was supported in part by a National Science Foundation Fellowship.

FIG. 1. a) Tobacco plants (variety 402) showing typical symptoms of black shank. b) Recovery of turgor in tobacco plants (1 and 2) cut off above the basal stem lesion and placed in water. Failure to regain turgor in plants (3 and 4) cut off below the lesion. These are the same plants shown in 1A.

nicotianae and sore shin, incited by *Rhizoctonia solani* Kühn. Finally fluids extracted from ground stem tissues of both healthy and black shank infected tobacco plants were introduced into stems of healthy plants and their effects upon the vascular system were studied.

THE EFFECT OF THE DISEASE ON WATER MOVEMENT IN INFECTED PLANTS.—*Recovery of turgor in diseased plants.*—Tobacco plants of the black shank susceptible 402 variety were grown in 6-in. pots until they were approximately 15 in. high. Each plant was then inoculated by placing inoculum, an agar block 1 × 1 cm. in size, 1 in. deep in the soil on each side of the plant, approximately 1 in. out from the base of the stem. Oatmeal agar cultures 2–4 weeks old were used as inoculum. This method of inoculation gave satisfactory results if the temperature remained above 80° F. and adequate moisture was supplied to the soil. Within 1 week after inoculation the plants were usually wilted and 2–4 days later lesions were visible on the basal portions of the stems. Four plants showing typical, severe wilt symptoms and with well developed stem lesions were selected for the first test (Fig. 1a). These plants had been wilted for 2 days. The plants were removed from the pots and the roots washed free of soil. The 2 plants shown on the left in the photograph were cut off, under water, above the basal stem lesion and the cut stems placed in water. The 2 on the right were treated similarly except that the stems were cut off below the lesion. Within 2 hours the 2 plants on the left had regained turgor, while those on the right remained flaccid regardless of how long they remained in water (Fig. 1b).

In similar tests it was found that diseased plants which had been wilted for 5–7 days regained turgor when cut off above the basal stem lesion and placed in water, but not so rapidly as those which had been wilted for a shorter period. In the case of plants which had been wilted for an extended length of time, the margins of the lower leaves were frequently necrotic, while the centers were chlorotic. When these plants were cut off above the lesion and placed in water, the chlorotic areas of the lower leaves, as well as the green upper portions of the plants, regained turgor. Only the necrotic areas were not affected by the acquisition of water. The plants which regained turgor and were kept in water several days not only remained turgid but frequently put out new roots. It was also found that wilted, diseased plants when left intact became turgid when placed in a moisture saturated atmosphere. However, the time necessary for recovery was somewhat longer than with plants which were cut off above the lesion and placed in water, usually varying from 8–12 hours.

Dye Movement Studies.—Eosin dye was used to trace the movement of water through plants. The root systems of both diseased and healthy plants were immersed in a 0.5 per cent aqueous solution of the dye and the plants placed in a bright location, but out of direct sunlight. Within 4 hours the dye was found to be distributed throughout the healthy plant. In the diseased plant it had moved into the portion of the root system which was still not invaded, but it had not moved past the stem lesion.

Recovery of plants with the pathogen still present within host tissue.—The possibility that a wilted, diseased plant could be made to recover turgor while the pathogen was active within its tissues was next investigated. This was thought desirable because the method of cutting the stem off above the lesion, as described in the first experiment with the excised plants, separated the top of the plant from the sphere of activity of the organism. It was therefore possible that recovery took place simply because the toxic substance was no longer being translocated to the upper portion of the plant. A technique was devised, therefore, which would permit introduction of water into the stem above the lesion without severing the stem. A plant was inoculated by the method indicated in the first experiment. After a lesion had developed which extended several inches up the stem and severe wilting had

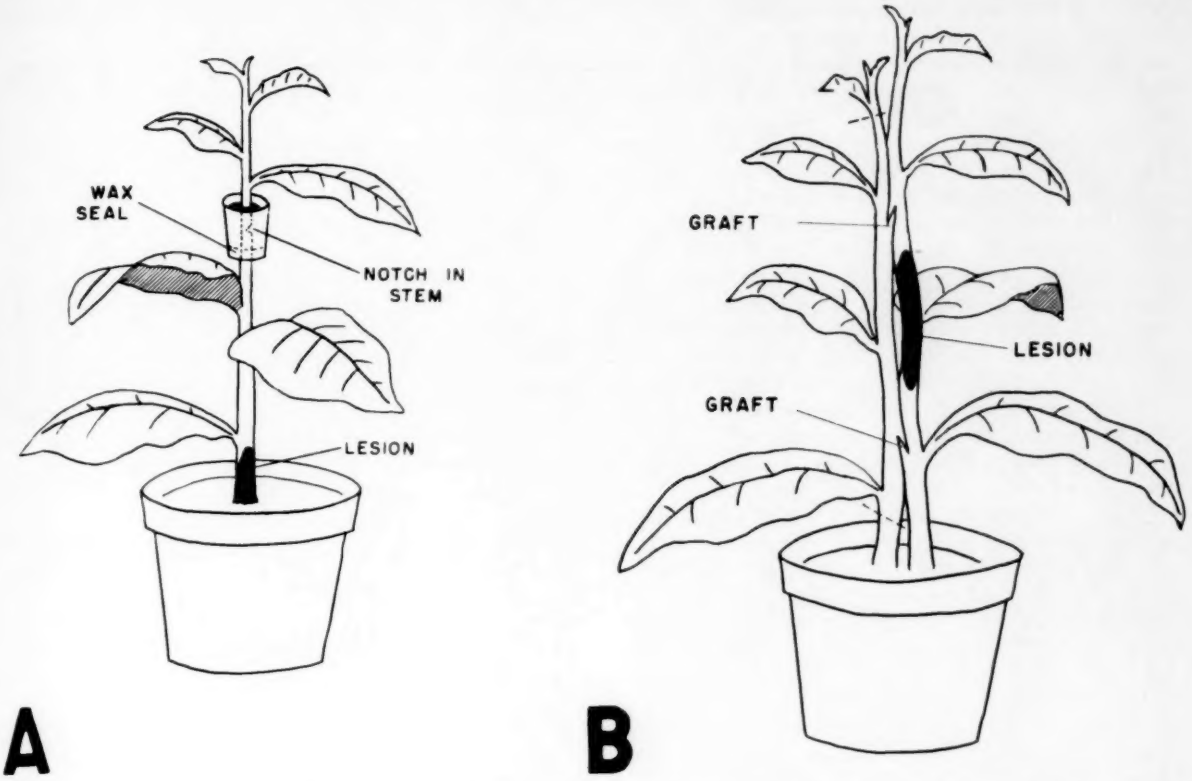

FIG. 2. a) A diagrammatic representation of a plant with a waterproof paper cup fitted around the stem. The plant recovered turgor when water entered the xylem through the notch. b) A diagrammatic representation of a bridge grafted plant. The plant on the right was inoculated with the black shank pathogen midway between the grafts. The dotted lines on the plant on the left indicate points at which the stem was excised.

occurred, a waterproof paper cup was fitted around the stem above the lesion as indicated in Fig. 2a. The cup was sealed at the bottom with paraffin wax and then filled with water. A notch was cut into the stem below the water level, thus allowing water to enter the xylem. As water was taken up by the plant, the supply in the cup was replenished to maintain the water level above the notch. After 3 hours, leaves above the notch had regained turgor, whereas those below had not. This experiment was repeated 3 times with similar results.

Bridge grafting was also used to determine whether plants were able to regain turgor while the pathogen was still present within the host. Two plants were grown in a single 6-inch pot until approximately 18 in. high. At that time 2 cleft grafts were made between the stems of the 2 plants. The lower graft was made 6 in. above the soil line and the upper graft about 6 in. above this. When a growth of abundant callus tissue around the graft indicated that a successful union had occurred, the stem of 1 of the plants was inoculated midway between the 2 grafts. Mycelium from an oatmeal agar culture of the pathogen was placed in a small slit cut into the cortex with a scalpel. The incision was covered with moist cheesecloth for 2 days to aid infection. After 1 week the lesion had completely girdled the stem and was approaching both the upper and lower grafts. At this time, however, the tops of both plants were turgid, even though one stem had a well developed lesion. The stem of the noninoculated plant was then severed just below the lower graft and above the upper graft, as indicated by the dotted lines in Fig. 2b. Thus a bridge of noninfected stem tissue was formed around the lesion on the inoculated stem. The infected plant was removed from the pot and the roots washed free of soil. The root system was then immersed in a 0.5 per cent aqueous solution of eosin dye. After 4 hours the plant was dissected and examined. It was found that the dye had moved into the roots and up the stem to the graft, through the bridge and back into the intact stem, but had not moved through the lesion. Other plants which had been similarly treated but which were not dissected for examination, remained turgid until the lesions extended to the grafts and therefore blocked the movement of water around the bridge of noninfected tissue.

Development of symptoms on diseased plants held in a moist chamber.—In a final experiment, plants were inoculated and immediately placed in a moist chamber in order to determine what symptoms would

develop in a moisture saturated atmosphere. The moist chamber was constructed of translucent plastic material and high humidity was obtained by introducing a fine spray of water through a special nozzle attachment.

Lesions were visible on these plants in about 5 days. This was several days sooner than on plants kept under ordinary conditions in the greenhouse. These lesions had the appearance of a soft-rot, rather than the firm, shrunken necrotic area typically produced by this organism. Under ordinary conditions the lesions usually extend up the stems for a distance of only about 4–6 in., but in the moist chamber they eventually advanced all the way to the top of the plant. Whereas the extent of the lesion increased under moist chamber conditions, the plants never wilted. As the lesion advanced up the stem and reached a leaf, the petiole decayed and collapsed, but the leaf lamina remained turgid.

These experimental procedures demonstrated that wilting of infected plants is due to a localized obstruction of water movement in the area of the lesion. In each case wilted plants regained turgor if water was introduced into the xylem above the lesion, or if the plants were placed in a moist chamber where it was possible for the leaf mesophyll cells to obtain water from a moisture saturated atmosphere.

HISTOLOGICAL STUDIES.—*Observations on fixed material.*—Material for histological study was taken from plants in 3 different stages of pathogenesis: 1) incipient wilting, 2) completely wilted with a lesion extending 1 in. above the soil line and 3) completely wilted with a lesion 3 in. above the soil line. Disks approximately ½ cm. thick were cut from the stems at the following points: 1) the top of the tap root, 2) the center of the lesion, 3) the advancing margin of the lesion, 4) at 1, and 5) 3 in. above the advancing margin. Material was also taken from healthy plants at the top of the tap root, the ground line, and 2 in. above the ground line. Each disk of material was cut radially into 8 sections and immediately placed in a formalin-aceto-alcohol fixative. The material was then dehydrated in a tertiary butyl alcohol series, and embedded in paraffin (9). Radial, tangential and cross sections, 12 microns thick, were made with a rotary microtome. The preparations were then stained with safranin and fast green and mounted with Permount.

Tissues invaded by the black shank pathogen.—All tissues of the stem within the lesion were found to be invaded by hyphae of the pathogen (Fig. 3a and 3b). The only essential difference between the sections made at the various stages of pathogenesis was the relative abundance of mycelium in the tissues, the tissue from older lesions being more completely invaded. The only cells which were never seen to be penetrated by hyphae were the protophloem fibers. These cells with thick secondary walls seemed to resist hyphal penetration. The parenchymatous cells of the cortex and pith were occupied to the greatest extent of any of the tissues. Mycelium was not found in the sections of material from the stem above the lesion.

Cells of the cortex in the lesion were collapsed and discolored. Both cells and intercellular spaces were usually occupied by mycelium. Cell walls were apparently penetrated at any point. The host-parasite relationship in the cortical cells was similar to that described by Nusbaum (7) in the cortex of roots of susceptible plants. When the hyphae first entered a cell, the protoplast was not visibly damaged. The cytoplasm then became vacuolate, indicating progressive impoverishment of the cell. Later the nuclei degenerated into disorganized masses which stained heavily with safranin. Finally the cell contents disappeared and the cell walls collapsed. There was no evidence to indicate that the causal organism destroyed the cells of the host in advance of the mycelium. This was the type of host-parasite relationship observed whenever the pathogen invaded parenchymatous tissue.

Phloem and cambium cells also were invaded and killed, and the collapse of these tissues was so pronounced that only the protophloem fibers retained their identity (Fig. 3a). All tissues in the xylem, the vascular rays, wood parenchyma and the vessel elements, were invaded. Vessels adjacent to the cambium and also those bordering the pith were most frequently invaded. The hyphae in the xylem primarily passed from cell to cell through the pits and showed conspicuous constrictions at these points. Once inside the vessels, the mycelium spread longitudinally, with many strands occupying a single vessel (Fig. 3d). The internal phloem was also invaded, but not to the extent of the external phloem.

The parenchymatous cells of the pith were heavily attacked. In this tissue, the mycelium advanced rapidly through the intercellular spaces and then subsequently branched out to penetrate adjacent cells. The upper margin of the lesion was most advanced in the pith, usually extending a short distance above the external margin. In the older portions of the lesion, the pith cells were collapsed and desiccated. This condition produces the discoid appearance of the pith, one of the characteristic macroscopic symptoms of the disease.

Presence of tyloses and gums.—Tyloses were found to be very numerous in the xylem elements. These sack-like protrusions from adjacent parenchymatous cells frequently completely filled the vessel elements (Fig. 3f). Tyloses in all stages of development were observed, from very small bud-stage forms to large, single sacks which filled the lumen of the vessel (Fig. 3e). Occasionally gummy masses of material were seen in the vessels which contained tyloses. Mycelium and tyloses were never seen together in the same cell, but they were frequently found in adjacent vessels. When a cross section of the stem was examined, vessels containing tyloses were seen to be more numerous than those with mycelium. Although the mycelium did not extend above the advancing margin of the lesion, tyloses were found as high as 1 in. above the lesion. In this region usually the small bud-stage forms were found with only a few large, well devel-

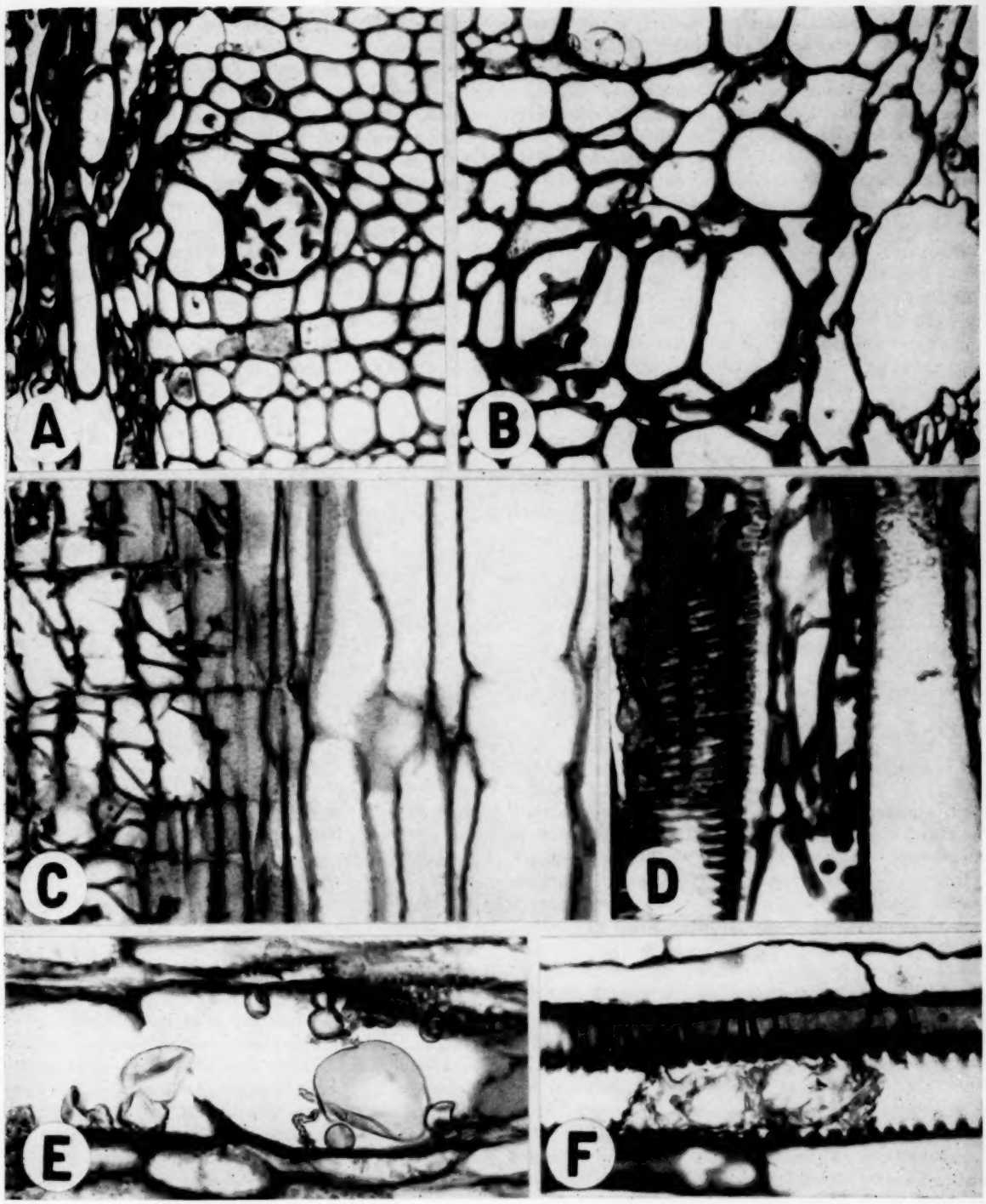

FIG. 3. a) A cross section through the basal portion of a black shank stem lesion showing infection in the cortex, phloem, cambium, and adjacent xylem. 380×. b) A cross section showing infection in the pith, vascular rays, and vessels. 380×. c) Longitudinal section of xylem from the lesion of a plant affected by sore shin. The mycelium is limited to the parenchyma cells of the vascular rays. 380×. d) Longitudinal section of infected xylem tissue with numerous strands of hyphae in the vessel elements. 380×. e) A single vessel element occupied by tyloses of several different sizes. 380×. f) A mass of tyloses within a single vessel element. 380×.

oped tyloses being present. The tissue 3 in. above the lesion was free of tyloses as well as mycelium, being indistinguishable from healthy tissue. Later studies of the pathological histology of black shank infections in younger, more succulent plants revealed the presence of tyloses and gums at even greater distances above the lesion. Frequently, in plants about 12 in. tall, the xylem was found to be discolored for more than ½ the height of the stem. Thus, whereas the mycelium remained confined to the lesion in all the plants examined, there was considerable variation in the extent of the gums and tyloses within the vessels.

Observations of tissue from healthy stems.—In material from healthy stems, which was examined as a check, neither tyloses nor gums were found. However, in some of the parenchymatous cells of the cortex and the pith, masses of granular material were seen. This material appeared to be different from the type of gummy substances found in the vessels containing tyloses. The material associated with the tyloses always stained deeply with safranin, whereas that in the parenchymatous cells did not.

Observations of fresh material.—All the observations made with the prepared sections could be confirmed by using free-hand sections of fresh material. It was noticed in free-hand sections of diseased stem tissue that the walls of xylem elements which contained tyloses were dark brown in color. These vessels contained conspicuous amounts of amorphous material. The fixation, dehydration, and staining of histological preparations evidently had removed the discoloration and some of the gums, because only an occasional vessel was seen to contain this type of substance in the stained sections.

The nature of the gummy material.—Since this gummy material was so abundant in the vessels of fresh preparations of diseased stem tissue and appeared to play an important part in pathogenesis, an attempt was made to determine the nature of the substance. Microchemical tests indicated that the material was probably a complex mixture of pectic and fatty substances. Some of the material was soluble in acetone and was saponified by a mixture of equal parts of saturated KOH and 20 per cent NH_4OH, diluted with 2 volumes of H_2O. Also, the material stained with ruthenium red at a concentration of 1:1,000, which is characteristic of pectic substances. Under polarized light, the gummy masses were isotropic, a characteristic of both fatty and pectic materials.

Studies on tylose formation.—Since tyloses were found to be so abundant in the vessels of diseased plants a study of the factors involved in tylose formation was made. The literature regarding the fundamental cause of tylose formation is contradictory. Neither of the 2 outstanding theories in regard to this subject were developed using diseased material. Haberlandt (4) considers that the decomposition products of injured cells (wound hormones) may play a decisive role in causing tyloses to form. This theory is based on the fact that tyloses are often found beneath the surface of amputated branches or adjacent to wounded areas. Klein (5) and Winkler (11) support a different interpretation of these observations. They consider the presence of air within the exposed vessels to be the chief cause of tylose formation. Klein conducted extensive experiments in order to substantiate this theory and concluded that the decisive factor in tylose formation was whether the parenchyma cells adjacent to the vessels bordered on water or on air. Thus if a vessel had ceased to conduct water and contained only air, tyloses would be formed. Klein's work dealt mainly with *Robinia pseudoacacia* L., but he also used some herbaceous species and obtained the same results in all cases.

To determine whether the technique used by Klein would also induce tylose formation in excised tobacco stems, the following experiment was conducted using tobacco plants (variety 402). Sections of stems about 12 in. long were placed in 1-liter flasks containing tap water. The upper 4 in. of the stems extended above the mouth of the flask and the cut surfaces were coated with paraffin to prevent exposure to the air. The controls were stems with the cut surfaces left untreated. After 2 weeks, these stems were examined microscopically, the slides being prepared by the paraffin method as described previously. In all the stems on which the cut surfaces were left exposed, tyloses were very numerous in the vessel elements of the first 2 cm. below the surface. In contrast, tyloses were not found in the stems with cut surfaces coated with paraffin. These findings agreed with those obtained by Klein, indicating that tylose formation may have been associated with the presence of air in the vessels.

To make a further test of this theory, a histological study of plants which had been inoculated and placed in a moist chamber was undertaken. As mentioned previously, although lesions developed more rapidly under these conditions, wilting did not occur. Therefore, it could be assumed that the water columns in the vessels remained intact and that there was no air in the vessels. Thus, the conditions which Klein states are necessary for tylose formation were not fulfilled.

Sections of diseased stem tissue were taken from plants in the moist chamber and histological preparations were made in the manner previously described. As noted in the histological preparations discussed earlier, the mycelium was limited to the lesion itself and tyloses were abundant. However, tyloses and gummy substances were found in sections taken 3 in. above the lesion, the uppermost sections studied. In addition, free-hand sections were also made at intervals up the stem to determine the extent of vascular discoloration. It was found that the vessels were discolored and contained gums up to within a few inches of the top of the plant. However, the amount of gum present within the vessels decreased with increasing distance above the lesions. The fact

that tyloses were found in this material, even though the conditions sipulated by Klein were not present, indicates that other factors also can be responsible for tylose formation.

Comparison of black shank, sore shin and Fusarium wilt infections.—In addition to the histological study of black shank infected plants, sections were made of material taken from plants with sore shin (*Rhizoctonia solani*), and with Fusarium wilt (*Fusarium oxysporum* var. *nicotianae*). The sections were prepared by the methods given previously. Sore shin causes a basal stem lesion which is very similar in outward appearance to the lesion produced by the black shank organism. With sore shin, however, the plants do not wilt suddenly as is the case in black shank. Wilting does not appear until the late stages of the sore shin disease. Due to weakness of the stem in the region of the lesion, plants are frequently broken at the base of the stem before wilting begins.

The sections of sore shin material, taken from the center of a well developed lesion, showed that the invasion of the xylem was largely limited to the parenchymatous cells of the vascular rays with the vessel elements usually remaining unobstructed (Fig. 3c). Only in a few sections were hyphae seen in the vessels and occasionally small tyloses were observed. The hyphae passed from cell to cell along the vascular rays through the pits in the cell walls. The cortex of the lesion was shrunken and dead, with the individual cells collapsed and pressed together so tightly that they appeared to be a disorganized mass of discolored material. The pith in the center of the lesion was completely disintegrated, leaving this part of the stem hollow. Material from above the lesion was not taken for examination; therefore, the distance that the mycelium extended up the infected stems was not determined.

Sections of material from the base of the stems of plants with Fusarium wilt showed very abundant mycelial development in the vessels. The hyphae were so numerous and intermeshed that the entire lumina of many vessels seemed filled with a tightly packed network of mycelium. Macrospores and chains of chlamydospores were also being produced within the vessels. Gums and tyloses were seen in occasional vessels, although they were less abundant than in tissue from black shank lesions. The parenchymatous cells adjacent to the vessels did not seem to be invaded to any appreciable extent. The pith, cortex and phloem also were not affected.

INDUCTION OF WILTING BY PLANT EXTRACTS.—In the first paper on the cause of wilting in black shank, Wolf (12) reported that extracts of diseased tissue would cause irreversible wilting of intact plants. Subsequently, Wolf found that sap from diseased plants would cause irreversible wilting of tomato and tobacco leaves (13).

Experiments were undertaken to study the effects of extracts of plant tissue on healthy plants. Tissue was taken from black shank lesions and from the lower part of stems of healthy plants. The cortex and pith of 12 stem sections 6 in. long were ground in a mortar and pestle. Since tissue from infected plants was somewhat desiccated, it was necessary to add a volume of water approximately equal to that of the tissue in order to obtain an extract. Tissue from the healthy stems was succulent and therefore it was not necessary to add water in this case. After the extracts were obtained, they were filtered through a Seitz bacteriological filter.

Succulent young plants (variety 402) about 12 in. high were used in this test. The stems were cut off under water and the cut ends placed in vials of the extracts. Half of the plants were placed in the extract from diseased tissue and half in the extract from healthy tissue. There was no visible effect on either group of plants during the first 6 hours. Within 12 hours, however, tips and margins of leaves on all plants were curled downward. The midribs and large veins seemed to remain turgid, whereas the leaf laminae were flaccid. After 24 hours the entire leaves showed pronounced wilting. During the second 24 hours the plants became even more flaccid. Forty-eight hours after the start of the experiment the plants were placed in bell jar moist chambers. After 12 hours in the moist chambers they showed pronounced recovery, but did not become completely turgid. When the plants were removed from the bell jars, they quickly wilted again.

After 72 hours a section 1 in. long was removed from the base of the stem of each plant, and the plants were then placed in fresh water. This treatment had no effect on the turgor of the plants. The 1–in. sections of the stems were examined microscopically. Many vessels of the xylem in the sections were discolored. Subsequent examination of the entire plants showed that these vessels were discolored throughout the length of the stem. The affected vessels were dark brown in color and contained both gums and tyloses. In examining cross sections of this material it was found that approximately ½ of the vessels were so affected. This discoloration, and the gums and tyloses were very similar to those found in plants affected by black shank. Except for the absence of mycelium, it was impossible to distinguish between a cross section of the xylem in this material and a cross section of xylem from a black shank lesion. Furthermore, the reactions to microchemical tests given by the gums in these sections were similar to those given by gums present in black shank lesions.

When the experiment was repeated using plants approximately 1 month older and much less succulent than those previously tested, some variation in symptomatology was noted. However, as in the first test, no difference was observed in the effects caused by the 2 different types of extracts. Leaf curling again was observed after the plants had been in the extracts for 24 hours; however, it was not nearly so severe as that found with the younger plants. Again the veinal tissue seemed to remain turgid whereas the leaf laminae became flaccid. After 36 hours the lower leaves were wilted while the uppermost leaves showed

only slight indications of wilting. After 72 hours in the extracts the entire plants were wilted.

When the sections from the base of the stems were examined some of the vessels were again found to contain tyloses and gums; however, they were much less abundant than in the younger plants. Sections taken at intervals up the stem revealed that the discoloration of the xylem was more pronounced in the upper portion of the plant. Also the gums and tyloses were more abundant in these areas, approaching that found in the plants used in the first test.

The older plants also absorbed much greater quantities of the extracts than did the young plants used in the first test. The young plants, which became severely wilted in the first 24 hours, did not absorb any appreciable amount after that time. The more mature plants continued to absorb the extracts until the third day, at which time the entire plant was wilted.

The xylem tissue of healthy plants with the stems cut off under water and held in water for 3 days were also examined. Neither gums not tyloses were found in any of these check plants which remained turgid and normal in appearance.

Discussion.—All the results obtained support the view that systemic wilting in black shank infected plants is caused by the localized obstruction of water movement through the lesions. The fact that tobacco plants affected by black shank, even after a prolonged period of wilting, were made to regain their turgor by the introduction of water into the stem above the lesion is significant. This capacity for recovery of turgor was not affected by the activity of the pathogen within host tissue, as was demonstrated by both the paper cup technique and bridge grafting.

These facts alone raise a question as to the validity of the assumption that wilting is due to the systemic action of a toxic metabolic product of the pathogen. If this assumption were correct, recovery of turgor would not occur after the onset of wilt symptoms. According to the concepts of toxigenic wilting in plants which have been developed by recent workers, toxic substances induce wilting by increasing the permeability or otherwise injuring the plasma membranes of the leaf cells (1, 2, 3). Once the plasma membranes are adversely affected an irreversible wilting develops because the osmotic system cannot function in the absence of a differentially permeable membrane. As the recovery of turgor depends upon the proper functioning of the osmotic system, any damage of this type would be critical.

Studies on dye movement, bridge grafting, and recovery of turgor in diseased plants indicated that black shank lesions obstructed the flow of water, but they did not reveal the factors which caused the xylem to cease functioning in water transport. It was not known to what extent the pathogen itself might be responsible, either directly by physically blocking the vessel elements with its mycelium, or indirectly by the injurious effects of its metabolic products on tissues within the lesion during pathogenesis. Moreover, the possibility of the xylem being blocked by the products of host cell degeneration in the lesion was considered. Studies of the pathological histology of black shank infections and of the phenomena associated with the formation of tyloses and the gummy materials in xylem tissues clarified these points.

Microscopic examination of diseased tissues from black shank lesions revealed the presence of gums and tyloses within the vessels as well as abundant masses of mycelium. In observing single cross sections of xylem tissue, it appeared at first that many tracheary cells were unaffected and that parts of the xylem, at least, might be functional. However, in examining serial sections involving several hundred microns of stem tissue, most of the vessels observed appeared to be obstructed at one point or another by either gums, tyloses or mycelium. The parenchyma cells of the vascular rays were also obviously unable to function normally in lateral movement of water. This condition of the xylem tissue, in which both vessel elements and vascular ray cells appeared to be unable to maintain normal activities, offers a reasonable explanation for the lack of water movement through the lesion.

Sore shin of tobacco, which also is characterized by the formation of a prominent basal stem lesion, presented a striking contrast to black shank with regard to xylary invasion. Whereas the parenchymatous cells were destroyed in sore shin lesions, the vessels remained practically free of mycelium, tyloses, and gummy substances even during late stages of pathogenesis. This may explain why sudden, severe wilting does not occur in this disease. In Fusarium wilt of tobacco, a disease in which moderate wilting occurs, many of the vessel elements were found to contain mycelium and some tyloses and gums. However, it was obvious that there was much less obstruction of the xylem in this case than in black shank lesions. This situation may possibly explain why wilting is so much more severe in black shank disease.

Work with tylose formation revealed that tyloses could be caused to form in tobacco stems by the use of Klein's technique. However, it was also seen that they formed even when the conditions which he stipulated as necessary were not present. This is an important point because if Klein's theory that tyloses are formed only as a result of air being adjacent to xylem parenchyma cells were correct, then tyloses could only be the result of a previous cessation of water movement and could not be a cause of blocking themselves. In the case of black shank, therefore, it is obvious that the numerous tyloses seen in the vessels are not due to the presence of air and it can be concluded that these tyloses, in conjunction with the gums, play a principal role in obstructing water movement.

Experiments with extracts from tissue of both healthy and diseased stems revealed that such liquids caused wilting, with gum and tylose formation. The gums and tyloses formed were very similar to those seen in black shank infected plants. These results indicate that a pathological condition may be induced

by the deleterious activity of decomposition products of ground tobacco tissue and that these toxic effects are not necessarily related to any specific substances found only in diseased tissues.

The effects of tissue extracts on excised plants also aided in the interpretation of the role of tyloses and gums in the obstruction of the xylem. When succulent plants were placed in these liquids they wilted within 24 hours and uptake of the extracts was sharply reduced after that time. The fact that these plants regained turgor when placed in moist chambers, but not when transferred to water after having the basal inch of the stems removed, is significant. This indicated that wilting was due to blocking of the vessels in the stems, rather than to any toxic action upon the cells of the leaf laminae. Moreover, this obstruction was not confined to the basal part of the stems, as subsequent examination showed that the xylem of these plants contained tyloses and gums even in the upper portions of the stem. Using older plants, complete wilting did not occur until the third day and the uptake of the extracts continued well into the second day. Examination of the lower parts of these stems revealed relatively small amounts of gums and tyloses, thus explaining the continued absorption of the solutions. However, the upper portions of these stems were found to contain abundant tyloses and gums, approaching the amounts found in the stems of younger plants. Therefore, it would seem that the xylem parenchyma of very succulent plants is particularly sensitive to the effects of the extracts.

The variation in symptomatology seen in plants affected by black shank supports this theory of the hypersensitivity of immature xylem tissue to decomposition products. Young, rapidly growing tobacco plants usually wilt suddenly and very drastically when they become infected. The wilting is usually complete before the lesion is visible on the stem above the soil line. Older, more mature plants which are much less succulent do not wilt this rapidly. Frequently wilting is not complete until the basal stem lesion has extended several inches up the stem.

Whereas all these conclusions indicate that wilting is caused by plugging of the xylem, it would not be correct to assume that toxic substances are not involved in pathogenesis. Toxic action undoubtedly is responsible for the formation of the obstructing materials. Moreover, the evidence presented strongly indicates that the toxic substances involved are a product of the decomposition of cells which have been invaded by the pathogen. These substances cause xylem parenchyma cells to form either tyloses or gums. This situation produces the obstruction which is primarily involved in causing the wilting. The toxic substances, therefore, are not systemic in their action, but have an effect which is primarily restricted to a localized region in the xylem. Also they are not necessarily products of fungal metabolism because it has been shown that cell decomposition products alone induce a similar reaction.

In view of these findings, the toxin theory, as it is usually interpreted, is not acceptable as an explanation of the cause of wilting in the black shank disease. The only explanation which is in full accord with the facts presented is that wilting is due to the blocking of water movement through the root and stem lesions.

PLANT PATHOLOGY
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LITERATURE CITED

1. GÄUMANN, E. 1951. Some problems of pathological wilting in plants. Adv. in Enzym. **11**: 401-437.
2. GOTTLIEB, D. 1943. The presence of a toxin in tomato wilt. Phytopathology **33**: 126-135.
3. GOTTLIEB, D. 1944. The mechanism of wilting caused by Fusarium bulbigenum var. lycopersici. Phytopathology **34**: 41-59.
4. HABERLANDT, G. 1923. Wundhormone als Erreger von Zellteilungen. Beitr. z. Allgem. Bot. **2**: 46.
5. KLEIN, G. 1923. Zur Ätiologie der Thyllen. Zeitschrift für Botanik **15**: 418-439.
6. NOLLA, J. A. B. 1928. The black-shank of tobacco in Porto Rico. Jour. Dept. Agr. Porto Rico **12**: 185-215.
7. NUSBAUM, C. J. 1952. Host-parasite relations of Phytophthora parasitica var. nicotianae in roots of resistant and susceptible tobacco varieties. (Abs.) Phytopathology **42**: 286.
8. POWERS, H. R. 1952. Water movement in tobacco plants affected by black shank. U. S. Dept. Agr. Pl. Dis. Rptr. **36**: 127.
9. SASS, J. E. 1940. Elements of botanical microtechnique. McGraw-Hill Book Company, Inc., New York and London.
10. TISDALE, W. B., AND J. G. KELLEY. 1926. A Phytophthora disease of tobacco. Fla. Agr. Exp. Sta. Bul. **179**: 159-219.
11. WINKLER, H. 1905. Botanische Untersuchungen aus Buitenzorg I. Über einen neuen Thyllenthypus nebst Bemerkungen über Ursachen der Thyllenbildung. Annales du jardin botanique de Buitenzorg, Series 2, **5**: 19-37.
12. WOLF, F. T. 1933. The pathology of tobacco black shank. Phytopathology **23**: 605-612.
13. WOLF, F. T. 1953. Toxin production in relation to tobacco black shank disease. (Abs.) Phytopathology **43**: 294.

ANTAGONISM IN SOIL BETWEEN MACROPHOMINA PHASEOLI AND SELECTED SOIL INHABITING ORGANISMS[1]

Don C. Norton

SUMMARY

The charcoal rot fungus, *Macrophomina phaseoli*, was paired with *Aspergillus flavus*, *A. rugulosus*, *A. terreus*, *Thielavia terricola*, *Trichoderma lignorum*, and *Bacillus cereus* at opposite ends of soil columns, and antagonism observed. *M. phaseoli* was inhibited by all other organisms in sterile, unamended and amended soils. *T. lignorum* and *T. terricola* were the only 2 fungi which invaded the charcoal rot fungus colony extensively. *T. lignorum* was the only fungus which grew for any appreciable distance into non-sterilized soil. Spore production was abundant. The hyphae of all fungi lost vigor rapidly and, with the exception of *A. terreus*, decreased in visible numbers after any given area was invaded, whether growing alone or paired in sterile soils.

Spore production of all fungi, except *M. phaseoli*, was abundant in sterile soil. Spore production of *T. terricola* and *T. lignorum* was not noticeably decreased when these fungi invaded a *M. phaseoli* colony.

To obtain a better understanding of root rot diseases, a knowledge of the interrelationships of the pathogen with other organisms in the soil is desirable. Our knowledge of antagonism between organisms *in situ* is extremely limited and it is not known whether the antagonism is the result of the influences of a few or many organisms. The present paper presents the result of studies on antagonism between *Macrophomina phaseoli* (Maubl.) Ashby[2] and selected soil inhabiting microorganisms.

MATERIALS AND METHODS.—With the exception of *Macrophomina phaseoli*, the organisms used in this study were common ones isolated from soil by the Warcup (6) method over a 1-year period from a field in which charcoal rot of guayule had been severe. These were *Aspergillus flavus* Lk., *A. terreus* Thom, *A. rugulosus* Thom & Raper, *Trichoderma lignorum* (Tode) Harz, *Thielavia terricola* (Gilman & Abbott) Emmons and *Bacillus cereus* Frankland & Frankland. The isolate of *M. phaseoli*, obtained from diseased guayule tissues, was the one used previously (4).

Antagonism was studied by placing 2 organisms at opposite ends of soil columns in glass tubes and observing, except with the bacterium, their growth toward each other. Duval fine sandy loam soil, adjusted to 5 per cent moisture level, was placed in the center 12 cm. of a glass column 45 cm. in length and an internal diameter of 1.3 cm. The soil columns were prepared by first inserting a plunger, made by wrapping gauze around a stick the diameter of a pencil, into the glass tube for about 16 cm. Soil was added to the other end until the center 12 cm. of the tube was filled. Each tube was gently tapped on a table during filling. A second plunger was inserted into the open end until it touched the soil in the tube. The apparatus was wrapped in newspaper and autoclaved for 2 hours. After cooling, 1 plunger was removed and a fungus disc 1.3 cm. in diameter, which had been rinsed in sterile water, was inserted aseptically into the tube so that the upper side of the fungus mat was in contact with the soil. The disc was previously taken from a 7-day-old colony growing on a yeast extract-dextrose solution. Five cm. of sterile quartz sand, 5 cm. of sterile soil, 3 ml. of sterile water, 5 cm. of sterile quartz sand, and a cotton plug were added in order. The tube was then reversed and the same procedure repeated, using the desired organism. When a bacterium was employed, 3 ml. of a spore suspension was poured into the tube. In 1 series, the soil was amended with ground guayule tissue comprising 13 per cent of the soil by weight. Similar tubes were prepared to measure linear growths of fungi alone in sterile soil, non-sterile soil, and sterile quartz sand.

After 1 day's growth, a zone of soil 1 cm. wide and extending in length to include all soil area not yet invaded by the fungi, was marked off by narrow paper strips so as to form a window. All observations were made in this area. The tubes were kept on their sides at 30°C. in the dark, except during observations. Recordings of linear growth and, except in guayule-amended soils, the number of visible hyphae measured across the window width along a line midway in the last day's growth were taken daily for a period of 9 days. With amended soils, profuse hyphal growth around the pieces of guayule tissue made accurate counts impossible. Observations on the abundance of fruiting bodies were also made. The data given are for the average of 3 tubes for each treatment. Each experiment was repeated twice with comparable results.

The "soil-sandwich" method of Hessayon (3), slightly modified, was adopted for measuring production of antibiotics. Fifty gm. of sieved, air-dried soil was moistened, placed in a Petri dish, and autoclaved for 2 hours. When the dishes were cool, 1.3 cm. fungus discs or 3 ml. of bacterial spore suspension, obtained as described previously, were added to them and incubated for 9 days in the dark at 30°C. The original fungus mats were then removed from the

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[2] The pycnidial stage of the isolate used was obtained by the method of Thirumalachar (5) but the conidia did not conform to those described. In view of the confusion existing regarding this organism, the conventional name of the charcoal rot fungus is retained.

plates and the soil was autoclaved for 2 hours. Ten ml. of yeast extract-dextrose agar was added. After the mixture had cooled, 10 ml. of cooled but not solidified agar was poured onto the soil-agar mixture. After 24 hours, a small uniform disc from a culture of *M. phaseoli* was placed in the center of each plate and the radial growth subsequently measured. Similarly treated soil, but to which no organism had been added was used as a check. There were 3 replicates to a treatment. The experiment was repeated with similar results.

Antibiosis was tested *in vitro* by making several pairings of the charcoal rot fungus with all oragnisms on yeast extract-dextrose agar in Petri dishes.

Results.—Nine-day linear growth of the fungi alone in sterile, unamended soil and in combination with *Macrophomina phaseoli* in sterile, unamended soil and in amended soils is represented graphically in Fig. 1–3. In sterile soil, *M. phaseoli* and *T. lignorum* grew rapidly, while the others grew slowly but steadily over the 9-day period.

In unamended soil, *M. phaseoli* completely arrested the linear growth of all fungi at or near the line of junction, except *T. lignorum*, whose growth rate was greatly retarded but never stopped during the period of observation. The charcoal rot fungus was clearly inhibited at the line of junction with all fungi where accurate observations could be made. The only uncertainty existed when the hyphae of *T. lignorum* invaded the growth zone of *M. phaseoli* for several millimeters and it was difficult to follow the hypae accurately. The charcoal rot fungus was greatly inhibited by the bacterium.

In amended soil, the linear growth of *T. lignorum* was inhibited only slightly by *M. phaseoli*. Although greatly inhibited by the charcoal rot fungus, *T. terricola* grew slowly for at least 21 days. The extent of invasion of *M. phaseoli* into amended soil occupied by hyphae of *M. phaseoli* growing from the opposite direction is somewhat uncertain but it was observed clearly that hyphae from opposite colonies did intermingle for several millimeters. As in the non-amended soil, there was little intermingling of the 3 species of *Aspergillus* with *M. phaseoli*. Sclerotia of the charcoal rot fungus were produced on the pieces of guayule tissue. None was observed beyond the line of junction with any opposing fungus.

Hyphal production and behavior.—Mycelium production in sterile quartz sand was very sparse after 2 days' growth. Production of hyphae in sterile non-amended soil was comparatively sparse (Table 1). In most cases, there was a decrease in number and vigor of visible hyphae, when a given day's growth was later measured. When the organisms were paired in sterile, non-amended soil, the production of mycelia was not noticeably different from that in soil where there were no pairings as long as invasion of one fungus colony by another did not occur. When *M. phaseoli* was paired with *B. cereus*, mycelium production was greatly retarded. However, it is not known to what extent the bacteria extended through the soil. When

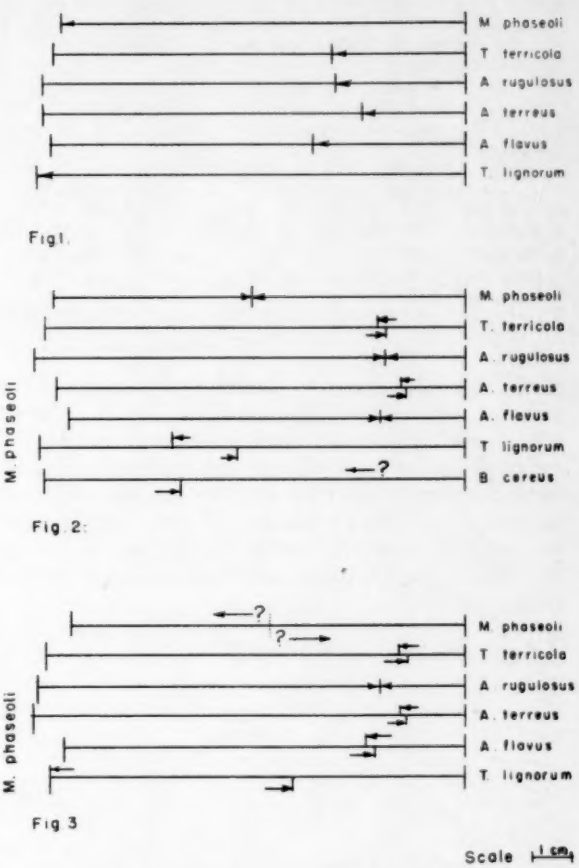

Fig. 1–3. Graphical representation of growth of various fungi and a bacterium, alone and in combination, in soil columns. Fig. 1. Extent of 9-day linear growth of fungi in unamended, sterile soil. Fig. 2. Extent of growth in unamended sterile soil, after 9 days, indicative of antagonism between *M. phaseoli* and selected soil-inhabiting microorganisms. Fig. 3. Extent of growth, in guayule amended sterile soil after 9 days, indicative of antagonism between *M. phaseoli* and selected soil-inhabiting microorganisms.

T. lignorum invaded a colony of the charcoal rot fungus, the number of hyphae of the former was greatly reduced and its growth rate was slowed considerably. Where there was no invasion of one fungus colony by another, linear growth of both fungi stopped abruptly at or near the line of junction. There was usually little hyphal contact between opposing fungi. After linear growth ceased, the hyphae rapidly lost their vigor and the number of visible hyphae decreased. Mycelium production was much greater in the amended than in non-amended soil.

Spore and sclerotium production.—All fungi except *M. phaseoli* produced fruiting bodies in sterile soil. The asexual stage of *A. rugulosus* was most abundant in the non-amended soil whereas the sexual stage was most abundant in guayule-amended soil. Fruiting bodies of the other fungi were, in general, most abundant in the amended soil. *T. lignorum* and *T.*

TABLE 1. *Visible hyphae of selected fungi in a 1 cm. band of soil at various days' growth and recounted at the end of the 9-day experiment*

Fungus	Hyphae near advancing edge of colony on day of observation					Hyphae visible after 9 days in area examined on day indicated				
	1	3	5	7	9	1	3	5	7	9
	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.
M. phaseoli	33	39	39	26	....	22	21	19	22	....
A. flavus	20	35	20	34	28	7	14	9	21	28
A. terreus	34	13	14	17	14	221	20	23	22	14
A. rugulosus	48	56	55	86	55	18	41	50	58	55
T. lignorum	130	67	55	....	....	?	36	23	....	....
T. terricola	188	128	163	145	145	98	117	136	153	145

terricola produced spores abundantly when growing with the charcoal rot fungus. Sclerotia of *M. phaseoli* were formed abundantly only on woody fragments.

Fungal growth in natural nonsterilized soil.—*T. lignorum* grew linearly 1.1 cm. through untreated soil in 3 days but failed to make any noticeable growth after that. Sporulation was abundant. The growth of the other fungi through untreated soil was almost negligible.

Production of antibiotics by soil microorganisms.—No antibiosis was noted when *M. phaseoli* was paired with the other organisms on yeast extract dextrose agar. Antibiotic production was not detected by the modified "soil-sandwich" technic.

DISCUSSION.—That sclerotia of *M. phaseoli* were not observed beyond the line of junction with *T. lignorum* and *T. terricola* is evidence that *M. phaseoli* did not invade colonies of these 2 fungi. The possibility must not be overlooked that hyphae of the charcoal rot fungus invaded the growth zones of the 2 other fungi but that sclerotium production did not take place. However, this seems doubtful because of the general inhibition of the charcoal rot fungus and because sclerotium production continued after *T. lignorum* and *T. terricola* invaded this fungus colony. Although mutualistic antagonism between *M. phaseoli* and several soil inhabiting fungi was demonstrated in non-amended soils, it is not known whether the inhibition is due to antibiosis or competition for substrate material. Antibiotic production *in vitro* of some of the fungi used has been reported. Due to the behavior of the hyphae near the point of junction of 2 opposing fungi, it is probable that the antagonism is not just a matter of a food-space relationship unless it is due to exoenzymes. That antagonism is not due to a concentration of gases is indicated by occurrence of the same phenomena when the organisms are similarly paired in sterile soil in partially filled Petri dishes. Chinn (2) recently found that 9 fungi originating from spores made very little growth into the surrounding non-sterilized soil. In the present study, since several common soil-inhabiting microorganisms inhibited *M. phaseoli* in the soil, it seems probable that the antagonism of this fungus *in situ* is due to the interaction of several organisms. This also could probably apply in many other cases as well. The material presented in this paper supports the concept that growth of fungi through soil is probably not so extensive as is often believed.

The fact that *T. lignorum* was the most competitive of the fungi employed might help explain the success in the use of this organism in reducing the severity of some plant diseases. Antibiotic production in amended, non-sterilized soil has been demonstrated with this fungus (7).

To the writer's knowledge no evidence has ever been presented that the charcoal rot fungus produces spores in the soil. It would seem that lack of spore production, along with its inability to compete with other soil microorganisms, would help account for the failure to isolate the fungus from the rhizophere (1, 4). Even though it is probable that most *Aspergilli* make little extensive growth through the soil, their abundant spore production results in frequent isolation. However, since so little is known about the activities of the *Aspergilli* in the soil and of spore production by fungi in general, the above is speculative.

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LITERATURE CITED

1. AGNIHOTHRUDU, V. 1953. Soil conditions and root diseases. VIII. Rhizophere microflora of some of the important crop plants of South India. Proc. Indian Acad. Sci. **37**: 1-13.
2. CHINN, S. H. F. 1953. A slide technique for the study of fungi and actinomycetes in soil with special reference to Helminthosporium sativum. Can. Jour. Bot. **31**: 718-724.
3. HESSAYON, D. J. 1953. Fungitoxins in the soil: II. Trichothecin, its production and inactivation in unsterilized soils. Soil Sci. **75**: 395-404.
4. NORTON, D. C. 1953. Linear growth of Sclerotium bataticola through soil. Phytopathology **43**: 633-636.
5. THIRUMALACHAR, M. J. 1953. Pycnidial stage of charcoal rot inciting fungus with a discussion on its nomenclature. Phytopathology **43**: 608-610.
6. WARCUP, J. H. 1950. The soil-plate method for isolation of fungi from the soil. Nature **166**: 117-118.
7. WRIGHT, J. M. 1952. Production of gliotoxin in unsterilized soil. Nature **170**: 673-674.

A RAPID SPECTROPHOTOMETRIC ASSAY FOR SOME PLANT VIRUSES [1]

Hugh C. Kirkpatrick and R. C. Lindner [2]

SUMMARY

A rapid spectrophotometric method for assaying the virus content of a stone fruit virus in cucumber seedlings based on increase in nucleic acid content is presented. This virus, which systemically invades cucumber plants, increased the nucleic acid content of cotyledons 25 to 35 per cent. The method has the advantage of ease and rapidity over earlier methods. More than 100 tests per day can be made, making possible a more rapid assay of virus content. The spectrophotometric virus titer increases with virus infectivity as measured by local-lesion counts. The spectrophotometric method has an advantage over a local-lesion assay because it is less influenced by growing conditions of the test plants.

Plant virus concentration has been measured spectrophotometrically after the isolation of the virus from the plant (4, 5, 8). Lindner et al (6) reported a simple spectrophotometric procedure for virus diagnosis, based on the ultraviolet absorption spectrum of an acid hydrolysate of leaf tissue, that does not require virus isolation. A method was needed for rapidly assaying the effect of chemotherapeutants on multiplication of viruses in plants with particular reference to a stone fruit virus in cucumber. This paper describes a modification of the spectrophotometric technique (6) for assaying the virus content of certain plant tissues.

METHODS.—Moore et al (7) noted that a virus from sour cherry foliage affected by necrotic ring spot was mechanically transmissible in juice to cucumber cotyledons. A virus causing symptoms in cucumber similar to those described by Moore et al has been recovered from peach and sweet cherry trees expressing one or another of a variety of stone fruit virus symptoms, and from mahaleb cherry and peach seedlings. The virus transmitted to cucumber may not have been the cause of any one of the symptoms expressed but may have been carried as a latent in the hosts. A culture of the virus transmitted to cucumber has been carried in cucumber and was used as the type culture for the work reported here. This virus will be referred to as the PLM virus (C-60 culture).

Chicago pickling cucumber seedlings in 2½-in. pots used as test plants were inoculated on the cotyledons before the primary leaf had begun to expand. Inoculum was prepared from infected cucumber plants inoculated 7–14 days previously. The tissue was homogenized in a blendor with 2–4 times the tissue weight of 0.01M neutral phosphate buffer. The cotyledons of test plants were dusted with 600 RA carborundum. Sterile gauze pads 12 layers thick were used for application of inoculum. Two strokes per cotyledon were accepted as sufficient.

For the spectrophotometric method described by Lindner et al (6), as modified for these experiments, ¼-in. discs of the cotyledons of each plant were selected as test tissue. The samples were cleared by 3 ten-minute washings in 70 per cent alcohol at 80° C. The samples were then hydrolyzed at 80° C. for 30 minutes in 3 ml. of alcohol and HCl (9:1 conc.) The hydrolysate was used for spectrophotometric analysis.

The hydrolysate absorption curves of both normal and diseased cucumbers peak at 2620 A. In essence, the method is based on the difference in total nucleic acid content of normal and PLM virus-affected tissues. The nucleic acid content is expressed by the optical density × 1000 at 2620 A.

Local lesions were determined by the starch-iodine test: a 35:1 ratio of 70 per cent alcohol to formalin was used for clearing cotyledons and IKI in lactic acid was used for staining (1 part of 2 per cent I and 6 per cent KI to 20 parts of lactic acid).

RESULTS.—*Variability in nucleic acid content of normal cucumber seedlings.*—To determine the variability in nucleic acid content of normal cucumber seedlings, 10 replicates of 1-disc samples were taken from each of 4 positions in the cotyledons (A_1, A_2, B_1, and B_2, Fig. 1), each across a major vein. Cotyledons A and B were distinguished by the direction of opening of the primary leaf. Another series of 50 plants was tested by taking a cotyledon disc from each of 2 plants per sample; and finally, lots of 50 plants each were tested using a cotyledon disc from each of 5 plants per sample. For these samples, tissue discs were taken from positions A_1 and B_2. The results are in Table 1. The 4 series represent samples from plants grown at different seasons of the year. The range in level of nucleic acid content is undoubtedly a consequence of this. The analyses suggest that variations between

FIG. 1. Sampling positions accepted as standard for cucumber cotyledon ultraviolet absorption tests.

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TABLE 1.—*Analyses of variance and mean levels of nucleic acid content in normal cucumber cotyledons under different sampling procedures, on a per disc basis*

Number of discs per sample	Variation	Degrees of Freedom	Mean Square	Mean nucleic acid content per disc [a]
1	Between plants	9	499.00	
	Between cotyledons within plants	10	1929.80	
	Between discs within cotyledons	20	720.95	225.2
2	Between sets of plants	24	1037.96	
	Between samples within sets	25	774.04	200.3
5	Between sets of plants	9	2516.11	
	Between samples within sets	10	1314.80	126.4
5	Between sets of plants	9	836.80	
	Between samples within sets	10	276.20	72.6

[a] Nucleic acid content expressed as optical density × 1000 at 2620 A. per disc.

plants, between cotyledons within plants, and between discs within samples must each be taken into consideration in deciding upon a sampling scheme. It is planned to pursue this point further. For the present it appeared most appropriate to use the 5-disc sample, the discs being taken 1 from each of 5 plants. Because of the marked variation due to season, it was also obvious that paired samples (inoculated and noninoculated) grown under the same conditions would be necessary.

Effect of PLM virus on the nucleic acid content of cucumber cotyledons.—During a 12-month period, 53 tests involving 10 or more plants per treatment were made to compare the ultraviolet absorption at 2620 A. of inoculated and noninoculated cucumber tissue extracts 4 to 7 days after inoculation. Absorption of the inoculated plant tissue extracts was 25 to 35 per cent greater than that of the non-inoculated plant tissue extracts. This consistently higher absorption is attributed to the increase in nucleic acid content brought about by the invading virus. The mean absorption per 5 discs of inoculated tissue was 799.0 while that of noninoculated tissue was 619.5. The mean increase in nucleic acid content of inoculated tissue was 179.5 ± 10.8.

The increase in nucleic acid content of inoculated tissue will be referred to hereafter as the spectrophotometric virus titer. It can be measured with an error of about 6 per cent, which is precise enough for most assay purposes.

Effect of numbers of tissue discs on absorbency.—The effect of the amount of cotyledon tissue used in preparing the hydrolysate for ultraviolet absorption was studied. In most preliminary experiments, 2 discs ¼-in. in diameter, 1 from each cotyledon, were taken from each plant. In 2 experiments the numbers of tissue discs per test were 1, 5, and 10. A third experiment included 1, 2, 4, and 8 tissue discs per test. The data are summarized in Fig. 2.

Increasing the number of tissue discs in the sample increased the ultraviolet absorption. There is a straight line relation for both inoculated and noninoculated tissue. It is obvious that the inoculated tissue extracts have a greater ultraviolet absorption at 2620 A. than do extracts of noninoculated tissue. When more than 5 discs are used per sample the results are erratic, possibly owing to incomplete extraction of nucleic acids. Five tissue discs have been adopted as the most convenient sample size.

Effect of cucumber cotyledon age on PLM virus concentration.—It has been the practice to inoculate cucumber cotyledons just as the primary leaf is unfolding. After the primary leaf has expanded to the point at which the width across the base is more than 2 in. the plants are not suitable.

To determine whether age of the cotyledon had an effect on PLM virus concentration, 3 age classes, based upon the basal width of the primary leaf at inoculation were established: no unfolded primary leaf (0), primary leaf trace to 1 in., and primary leaf 1 to 2 in.

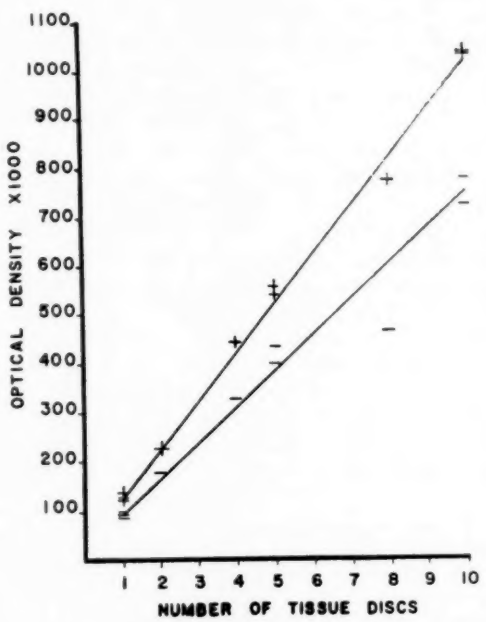

FIG. 2. The ultraviolet absorption of the hydrolysate as affected by increasing numbers of discs of inoculated (+) and noninoculated (—) tissue in the samples.

TABLE 2.—*Nucleic acid content of cucumber cotyledons of 3 age groups 6 days after inoculation*

Primary leaf size at inoculation [a]	Mean Relative Nucleic Acid Content per 5 Discs [b]		
	Noninoculated	Inoculated	Virus Titer
0	735	803	68
Trace-1	650	745	95
1-2	618	705	87

Analysis of variance

	Degrees of Freedom	Mean Square	*Ratio*
Treatment	1	1,809.67	7.948**
Size	2	1,359.25	5.969**
Replication	4	219.50	-----
Error	22	227.70	

[a] Age of cotyledon determined by width of primary leaf base in in.
[b] Nucleic acid content expressed as optical density × 1000 at 2620 A.
** P greater than 0.01.

Inoculated and noninoculated plants of these 3 age groups were tested 6 days after inoculation. The difference between the inoculated and the noninoculated was similar for the 3 age groups (Table 2). The difference in the absorption of the 3 age groups was mathematically significant at the 1 per cent level. Absorption of the hydrolysate from older plants is less than that from younger plants; however, the absorption of plants of the 3 age classes in the inoculated series was greater than that of those in the corresponding noninoculated series. The increased absorptions in the inoculated series are mathematically significant at the 1 per cent level.

Moore et al (7) reported that inoculated cotyledons persisted on the plant for some time after noninoculated cotyledons had withered. Whether the absorption difference might be due to the tissue thickness taken in the test disc was determined by taking 5 samples from each of the 3 age groups, which were ovendried at 80° C. for 6 hours and weighed to 0.1 mg. on a projection-reading analytical balance. The higher absorbency of infected tissue was not consistently related to larger sample weight. The mean weight of inoculated tissue was 1.46 mg. and that of noninoculated tissue 1.40 mg. Mathematically this difference was not significant at the 5 per cent level.

Effect of inoculation injury on nucleic acid content.—Tests were made to determine the effect of wounding, which occurs with mechanical inoculation of plants, on the nucleic acid content of cucumber cotyledons. Treatments included inoculating in the usual manner, rubbing with a gauze pad dipped in sterile water, and no treatment; and these 3 treatments were allocated to paired cotyledons in the 3 possible combinations. Each plant was tested individually 6 days later. In another series of tests the plants were tested 4 and 9 days after inoculation. Results are in Table 3. It is evident that rubbing or inoculation injury to the cotyledon has no significant effect on the nucleic acid content.

TABLE 3.—*Effect of rubbing injury on nucleic acid content of cucumber cotyledons*

Days after rubbing	Mean Nucleic Acid Content per 5 Discs [a]			
	Inoculated	Rubbed	Not Treated	L.S.D. at .05
4	530	472	480	32
6	497	386	370	61
9	530	322	308	95

[a] Nucleic acid content expressed as optical density × 1000 at 2620 A.

Effect of shading after inoculation.—To test the effect of absence of light, shading, and natural light, inoculated and noninoculated plants were covered immediately after inoculation with a shade that excluded natural light. Another set of plants was shaded so that they received no direct light. A third series adjacent to the shaded series was exposed to natural light.

The inoculated plants in the darkened series, when tested spectrophotometrically 4 days after inoculation, showed little to no build-up of virus titer. The spectrophotometric virus titer for the natural-light series was 161.3, that for the shaded series 62.5, and that for the darkened plants was 12.5.

To determine the effect of shading after the virus became established in the tissue, plants were inoculated 6 days before shading. At the end of the 6-day period the symptoms were well developed; all plants were tested, and half of them were shaded. Plants from the shaded series and those exposed to natural light were again tested after 24 hours. The total nucleic acid content of the plants in the shaded series decreased markedly as compared with that of the plants in the natural-light series (Fig. 3). The virus

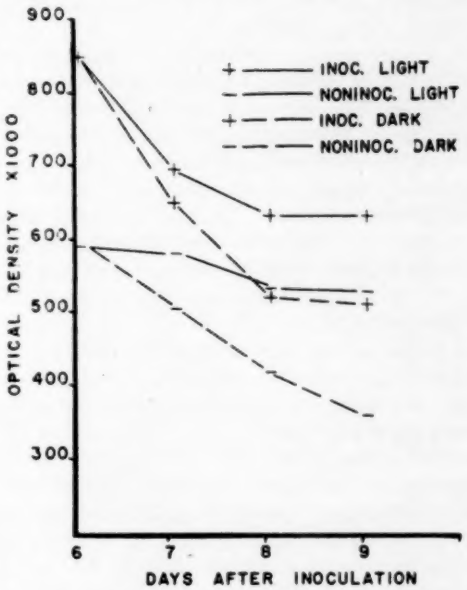

FIG. 3. The ultaviolet absorption of the hydrolysate from inoculated (+) and noninoculated (—) plants and the spectrophotometric virus titer showing the effect of shading 6 days after inoculation.

TABLE 4.—*Spectrophotometric virus titer of, and local-lesion counts on, cucumber cotyledons inoculated with crude-sap inoculum of different dilutions*

Dilution	Experiment 1		Experiment 2		Experiment 3	
	Virus Titer[a]	Local Lesions[b]	Virus Titer	Local Lesions	Virus Titer	Local Lesions
1:4	182.5	29.4	—	—	—	—
1:5	—	—	104.5	52.2	—	—
1:8	186.0	24.5	—	—	—	—
1:10	—	—	99.5	37.3	—	—
1:12	—	—	—	—	121.5	28.7
1:16	190.5	17.9	—	—	—	—
1:20	—	—	88.5	26.8	—	—
1:24	—	—	—	—	110.0	19.7
1:32	192.0	12.5	—	—	—	—
1:40	—	—	93.0	24.0	—	—
1:48	—	—	—	—	129.0	9.8

[a] Virus titer expressed as the mean increase in optical density × 1000 at 2620 A.

[b] Mean number of lesions per cotyledon.

titer decreased in both the light and shaded series from the 6th to the 9th day, but shading apparently did not reduce the relative amount of virus present. It would seem that light is important for virus increase during the infection, but once the virus is established, shading has little effect on virus content.

Effect of inoculum dilution on virus titer.—Three different series of dilutions of crude sap from PLM infected cucumbers were used as inoculum on cucumber plants in a study of the effect of inoculum dilution on virus titer. Local-lesion counts and the spectrophotometric analysis were made 6 days after inoculation. The local-lesion counts gave a measure of the virus activity in the inoculum, whereas the spectrophotometric virus titer was a measure of the systemic build-up of the virus 6 days after inoculation. Results are in Table 4. In any one experiment there was no significant difference in virus titer regardless of the number of original local lesions produced by different dilutions of inoculum. The conclusion was that within 6 days following inoculation the cotyledons became uniformly invaded by virus even though the initial inoculum varied in concentration. It will be noted that there is variation in virus titer among the 3 experiments. This is probably due to the fact that the experiments were performed at different times under different growing conditions.

Relation between virus titer and infectivity.—To determine whether virus titer as measured by the spectrophotometer is a true measure of virus quantity, a series of inoculated cucumber plants was tested at 24- or 48-hour intervals following inoculation. Tissue discs were removed for spectrophotometric testing. The remainder of the cotyledon was used for inoculum at 1:10 dilution for determining concentration by local-lesion counts on another series of plants. Three such series of test (1 in January, 1 in February, and 1 in March) were made. The relation between the spectrophotometric virus titer and the local lesions at the 1:10 dilution is shown in Fig. 4.

It is evident that with the increasing infectivity of the inoculum there is a marked increase in the spectrophotometric virus titer of that tissue. It will be noted, however, that infectivity (as determined by local-lesion counts) is markedly affected by growing conditions of the test plants. The 3 tests summarized here were conducted during a period when relative natural sunlight intensity increased from 1.00 to 3.16. (Sunlight was measured from black-bulb-thermograph readings during the period of infection.) It would thus appear that the spectrophotometric virus titer is a more uniform assay of virus content than are local-lesion counts, since the growing conditions of the test plants affect the development of local lesions and introduce a factor not concerning the initial inoculum titer.

DISCUSSION.—Much of the literature concerning the activity of virus particles attaches considerable importance to the relation of the nucleic acid constituent to biological activity (1, 2, 3). The method described here is a simple comparative measure of the nucleic acid content of tissue samples of noninoculated control and inoculated cucumber cotyledons by a spectrophotometric procedure. Since nucleic acid is a constituent of normal plants which varies in amount with growing conditions, all experiments must include noninoculated controls for comparison. In order that the results may be valid, uniformity in selection of the test tissue must be observed. In Fig. 1 it was indicated that the sampling site includes a major vein in the cotyledon. The virus with which this work was done causes initial local lesions followed by secondary systemic invasion of the cucumber plants. The inclusion of a portion of a major vein has been considered to be a means for obtaining a more uniform sample.

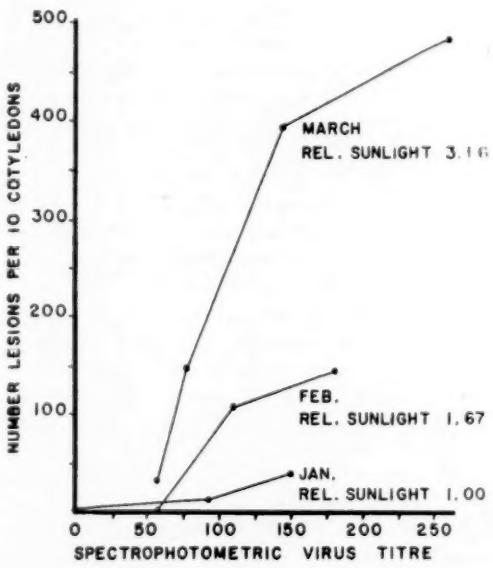

FIG. 4. Relation between the spectrophotometric virus titer of inoculum and infectivity measured by local lesions at intervals after inoculation, and at different times of the year.

This method has the advantage of rapidity and facility over previously reported methods (4, 5, 8) for which it is first necessary to isolate the virus from the sample. One hundred tests per day can readily be made, and thus a screening program for virus assay especially useful in the study of chemotherapeutants is facilitated.

The work reported has been limited to studies on a stone fruit virus (PLM) mechanically transmissible to cucumber. The method should be suitable for any mosaic-type virus in a host in which the virus is well distributed.

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LITERATURE CITED

1. BAWDEN, F. C. 1950. Plant viruses and virus diseases. Ed. 3. Chronica Botanica Company. Waltham, Mass. 327 p.
2. CLAUDE, A. 1943. Distribution of nucleic acids in the cell and morphological constitution of cytoplasm. In Biological Symposia **10**: 111-129.
3. KNIGHT, C. A. 1949. Constituents of viruses. In Ann. Review of Microbiology **3**: 121-136.
4. KUTSKY, R. 1952. Effects of indolebutyric acid and other compounds on virus concentration in plant tissue culture. Science **115**: 19-20.
5. KUTSKY, R., AND T. E. RAWLINS. 1950. Inhibition of virus multiplication by naphthalene acetic acid in tobacco tissue cultures as revealed by a spectrophotometric method. Jour. Bact. **60**: 763-766.
6. LINDNER, R. C., H. C. KIRKPATRICK, AND T. E. WEEKS. 1952. Ultraviolet absorption spectra as a tool for diagnosing plant virus diseases. Science **115**: 496-499.
7. MOORE, J. D., J. S. BOYLE, AND G. W. KEITT. 1948. Mechanical transmission of a virus disease to cucumber from sour cherry. Science **108**: 623-624.
8. TAKAHASHI, W. N. 1951. Ultraviolet absorption as a measure of tobacco-mosaic virus nucleoprotein. Phytopathology **41**: 142-145.

STUDIES CONCERNING CHEMOTHERAPY OF TWO PLANT VIRUSES [1]

Hugh C. Kirkpatrick and R. C. Lindner [2]

SUMMARY

Certain compounds were introduced by vacuum infiltration into cucumber plants inoculated the preceding day with a stone fruit virus (PLMV) and into tomato seedlings inoculated with tobacco mosaic virus (TMV); virus content was assayed 4–11 days later by spectrophotometric and biological methods. As measured spectrophotometrically, the PLMV content was markedly inhibited by chloramphenicol, thiouracil, or guanazola; as measured by numbers of local lesions produced, it was even more markedly inhibited by these compounds. The spectrophotometric titer of TMV was reduced by chloramphenicol, thiouracil, or guanazola treatment, but not by a treatment with malachite green.

Stoddard (16) indicated that X-disease virus of peach can be inactivated in dormant, diseased peach buds by certain chemicals. Takahashi (15) reported that malachite green inhibits development of tobacco mosaic virus in floating leaves. Commoner and Mercer (2, 3, 4) and Kutsky and Rawlins (7) used the floating leaf technique or liquid culture of plant tissue to study chemotherapy effects. Matthews (10, 11) studied chemotherapy effects of compounds sprayed on leaves, and Beale and Jones (1) utilized the wick method of introducing chemicals into plants. In 1948 the State College of Washington and the U. S. Department of Agriculture initiated a cooperative study of chemotherapy for control of stone fruit virus diseases. An early phase of this study was the development of an ultraviolet absorption technique for diagnosing plant virus diseases (9) and the modification of this procedure for assaying mosaic-type viruses (6).

This paper describes a vacuum infiltration method of applying compounds to cucumber plants previously inoculated with a stone fruit virus (PLMV) or to tomato seedlings inoculated with tobacco mosaic virus (TMV). The inhibitory effect of the chemotherapeutant was measured spectrophotometrically and biologically. The degrees of virus inhibition by some compounds are reported.

METHODS.—A stone fruit virus (PLMV) causing symptoms in cucumber similar to those described by Moore et al (13) has been recovered from peach and sweet cherry trees and from mahaleb cherry and peach seedlings. A stock culture from one of these sources has been carried in cucumber and was used for the work with cucumber seedlings. Tobacco mosaic virus (TMV) in tomato seedlings was also used in the study.

Inoculations were made on the cotyledons of Chicago Pickling cucumber plants before the primary leaf was fully expanded and on the terminal leaflet of the basal leaf of Early Wonder tomato plants before

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more than 3 leaves had unfolded. All chemical treatments were made 24 hours after inoculation.

The treatment procedure was vacuum infiltration of the foliage. The plants were inverted over beakers in a desiccator with the foliage immersed in the solution being tested. A vacuum of approximately 127 mm. of mercury was applied for 3 minutes when cucumber plants were treated and for 5 minutes when tomato plants were treated. Upon release of the vacuum the intercellular spaces of the cucumber cotyledons and the tomato leaflets were filled with the test solution, as indicated by water soaking. The plants were then placed on the greenhouse bench and watered only from the bottom until sampled.

Forty plants were used to test a single dilution of a chemical. These were divided into 4 lots of 10 plants each. Two lots were inoculated. One of the inoculated lots was subjected to chemical treatment (IT) while the other inoculated lot was retained as a check on the effect of the chemical on virus multiplication (I). One of the noninoculated lots was subjected to chemical treatment (T) while the second noninoculated lot was not treated to serve as a check on the effect of the chemical treatment on the plant (C). Thus for each lot that was both inoculated and treated (IT), there were 3 comparison lots: inoculated only (I), treated only (T), and healthy controls (C).

The assay procedure was the modified spectrophotometric analysis described by Kirkpatrick and Lindner (6). The ultraviolet absorption of an acid hydrolysate of the plant material was determined at 2620 A. Two samples were taken for spectrophotometric analysis from each lot of 10 plants. Each sample was a composite consisting of ¼-in. tissue discs from the cotyledon of each of 5 cucumber plants or from the terminal leaflet of the fourth developed leaf of tomato plants. The cucumbers were sampled 4–6 days after inoculation and the tomatoes were sampled 8–11 days after inoculation.

Previous work (6) indicated that the difference in absorption at 2620 A between lots I and C was a measure of virus titer. The difference between IT and T therefore represents the virus titer in treated plants. The virus titer in untreated plants varies with growing conditions and time; therefore, to obtain comparative data, lots I and C were included in every experiment. For the purpose of comparison we use the term "percentage inhibition in virus titer." This means the virus titer of untreated plants minus the virus titer of treated plants divided by the virus titer of untreated plants.

EFFECT OF WATER INFILTRATION ON ULTRAVIOLET ABSORPTION OF TISSUE.—A preliminary study of the effect of the vacuum infiltration upon inoculated and noninoculated cucumber plants was made using water as the treatment. It was evident that water itself was not responsible for any large difference in readings on treated and untreated plants. The water infiltration series had an absorption difference which varied from 1.5–14.0 per cent greater than the check series.

EFFECT OF INFILTRATION OF CHEMICALS ON SPECTROPHOTOMETRIC VIRUS TITER IN CUCUMBER.—In initial screening tests chloramphenicol[3], thiouracil, and guanazola[3] appeared to limit PLMV titer in cucumber. Therefore, further studies were made with these materials.

Chloramphenicol.—Chloramphenicol is reported to be active against a variety of Gram-positive and Gram-negative bacteria. It has also been reported to be effective against such rickettsial diseases as Rocky Mountain spotted fever, typhus, typhoid fever, and Japanese scrub typhus (12).

Beale and Jones (1) reported that 0.2 per cent solutions of chloramphenicol when introduced into *Nicotiana tabacum* L. test plants by means of wick feeding previous to inoculation had no effect on development of tobacco mosaic and potato yellow dwarf viruses.

In tests involving PLMV, 0.01–0.0005 per cent chloramphenicol was applied 24 hours after inoculation. The single application inhibited the virus multiplication as determined spectrophotometrically. This inhibitory effect diminished with lower concentrations of the chemical (Table 1).

A local lesion assay was made comparing lot I and the 0.01 per cent dilution of chloramphenicol, lot IT. The cotyledons tested for spectrophotometric virus titer were homogenized at the rate of 1 part of cotyledon fresh weight to 25 parts of neutral 0.01 M phosphate buffer by volume. A single cotyledon on each of 40 healthy cucumber plants was inoculated with the I homogenate. The opposite cotyledon was inoculated with the IT homogenate, thus giving a paired cotyledon assay. Whereas the spectrophotometric assay indicated a virus inhibition of 61.7 per cent, the local lesion assay indicated an inhibition in infectivity of 81.8 per cent.

Thiouracil.—Commoner and Mercer (2) pointed out that thiouracil inhibits tobacco mosaic virus biosynthesis in isolated disks of tobacco leaf tissue. At 0.0001 M concentration of thiouracil, floating leaf tissue formed 40 per cent as much tobacco mosaic virus as untreated tissue (3).

Thiouracil, which is only slightly soluble in water, was disolved in 0.001 M K_2HPO_4 for the work con-

[3] We are indebted to Parke Davis Co. for chloramphenicol, and to the Lederle Laboratories Division of the American Cyanamid Co. for guanazola.

TABLE 1.—*Inhibiting effect of chloramphenicol on PLMV in cucumber*

Chloramphenicol concentration (per cent)	Spectrophotometric virus titer I-C	IT-T	Percentage inhibition
0.01	209.0	80.0	61.7
0.005	252.0	90.0	64.4
0.002	280.0	150.0	46.4
0.001	280.0	155.0	44.6
0.0005	280.0	212.5	24.1

TABLE 2.—*Inhibition of PLMV in cucumber by 0.001 M thiouracil*

Spectrophotometric virus titer I-C	IT-T	Percentage inhibition
163.5	118.5	27.5
211.0	145.0	31.3
300.0	174.8	41.7
282.0	130.0	53.9
238.5	95.0	60.2
278.0	63.5	77.2

ducted here. A series of treatments at different times of the year with 0.001 M thiouracil resulted in variable inhibition of virus multiplication (Table 2). No reason for this wide variation has been determined.

Treatments with 0.001–0.004 M concentrations of thiouracil progressively inhibited PLMV (Table 3). This increase, however, was not linear. Whereas cucumber plants treated with 0.004 M thiouracil continued to grow and expanded primary and secondary leaves, injury from the treatment was apparent in stunting and chlorosis of both noninoculated and inoculated plants.

Infectivity of the homogenate from I and IT (0.001 M thiouracil) cucumber plants was compared with the spectrophotometric virus titers. The 3 homogenate dilutions were 2:1, 1:5; and 1:10 fresh weight to 0.01 M neutral phosphate buffer. Infectivity was inhibited by at least 76.1 per cent (Table 4). Virus inhibition as determined by the spectrophotometric virus titer was 27.5 per cent.

The difference between the infectivity inhibition and inhibition spectrophotometrically determined is marked, but, as with chloramphenicol treatment, the infectivity inhibition is greater.

Guanazola.—Kidder et al (5) found that guanazola administered to rats checked tumor growth. This inhibition is indicated as being affected through the substitution in nucleoprotein of the guanine analogue guanazola. Nickell et al (14) found that virus tumors of *Rumex acetosa* L. on nutrient agar were inhibited by guanazola at all levels tested down to 0.001 mg./ml. Matthews (10, 11) found guanazola to inhibit symptom expression and infectivity of a strain of lucerne mosaic virus in tobacco plants when guanazola was applied by spraying before and after inoculation or when applied to the soil. Two other triazolopyrimidines were tested, but guanazola was the only one with substituent groups in the same position as in guanine. This fact

TABLE 3.—*Inhibition of PLMV in cucumber by thiouracil concentrations of 0.001 to 0.004 M*

Concentration	Spectrophotometric virus titer I-C	IT-T	Percentage inhibition
Test 1:			
0.001 M	282.0	130.0	53.9
0.002 M	282.0	40.0	85.8
Test 2:			
0.001 M	300.0	174.8	41.7
0.002 M	300.0	32.5	89.2
0.004 M	300.0	25.0	91.7

TABLE 4.—*Infectivity of 3 dilutions each of the homogenates and spectrophotometric virus titer of control and thiouracil-treated plants*

Homogenate dilution	Local lesions (Average 30 cotyledons) I	IT	Percentage inhibition	Spectrophotometric titer I-C	IT-T	Percentage inhibition
2:1	26.05	6.25	76.1			
1:5	2.55	.30	88.2			
1:10	0	0	.....			
				163.5	118.5	27.5

supports the view that guanazola may act by replacing guanine in the virus nucleoprotein. Matthews (11) in further work with lucerne mosaic virus found that adenine, guanine, and hypoxanthine at 0.01 M concentration reversed the activity of guanazola (0.005 M in 0.1 per cent sodium bicarbonate). His results suggested that lucerne mosaic virus and possibly tobacco mosaic virus may utilize free adenine and guanine and that hypoxanthine may be a precursor of guanine and perhaps adenine in normal virus synthesis. It is possible that guanazola may interfere with the incorporation of guanine and perhaps adenine in the virus or actually replace them.

In a series of tests, dilution ranges of guanazola from 0.5 per cent to 0.0005 per cent were made (Table 5). In all tests with guanazola there have been no indications of phytotoxicity to cucumber.

In a paired cotyledon infectivity test, the homogenate of IT (0.05 per cent guanazola) cotyledons was tested on 1 cotyledon of each of 30 plants. The remaining cotyledons were inoculated with a homogenate of I cotyledons. Dilutions used were 1:10 and 1:30 of cotyledon fresh weight to neutral 0.01 M phosphate buffer. There was a single local lesion from the 1:10 dilution and no lesions from the 1:30 dilution indicating nearly 100 per cent inhibition of infectivity. The spectrophotometric virus titer inhibition was 89.4 per cent.

RESULTS WITH TOBACCO MOSAIC VIRUS (TMV).—Tobacco mosaic virus has been used in a number of chemotherapy studies. Takahashi (15) reported the inhibition of tobacco mosaic virus in tissue discs of *Nicotiana glutinosa* L. floating on malachite green solutions of concentrations as low as 2 ppm. Commoner and Mercer (3) floated inoculated leaf tissue on *N. tabacum* on half-strength Vickery's solution

TABLE 5.—*Inhibition of PLMV in cucumber by guanazola concentrations from 0.5 per cent to 0.0005 per cent*

Test	Guanazola concentration (per cent)	Spectrophotometric virus titer I-C	IT-T	Percentage inhibition
1	0.01	175.5	47.5	72.9
	0.001		93.0	47.1
2	0.01	230.0	165.0	28.3
3	0.05	117.5	12.5	89.4
	0.01		82.5	29.8
	0.0005		107.5	8.5
4	0.5	142.5	—5.0	103.5

TABLE 6.—*Effect of 0.001 M thiouracil, 0.001 M malachite green, 0.001 per cent chloramphenicol and 0.0005, 0.01, and 0.05 per cent guanazola on spectrophotometric virus titer of TMV in tomato plants*

Treatment		Spectrophotometric virus titer	Percentage inhibition
Control		397.0	
Chloramphenicol	0.001%	236.5	40.4
Thiouracil	0.001 M	31.5	92.1
Control		272.5	
Malachite green	0.001 M	261.2	4.1
Thiouracil	0.001 M	11.3	94.7
Control		265.0	
Guanazola	0.005%	100.0	62.3
	0.01	145.0	45.3
	0.05	65.0	75.5

containing various concentrations of thiouracil and reported complete inhibition of TMV synthesis at a concentration of 4.3×10^{-5} M. Leben and Fulton (8) used detached leaves, inoculated side up, on an agar medium containing test substances and found that terramycin inhibited infectivity of TMV.

To determine whether chemicals would react with other viruses as they did with PLMV in cucumber, tomato seedlings inoculated with tobacco mosaic virus were tested. Tomato seedlings require about 5 minutes of treatment to get good evacuation as evidenced by the water soaking of the foliage when returned to normal pressure. The terminal leaflet of the fourth developed leaf was selected as the site for the tissue test.

In 2 tests in which 0.001 M thiouracil in 0.001 M K_2HPO_4 was used, there was greater TMV inhibition than had been evidenced with PLMV at this concentration (Table 6). Chloramphenicol at 0.001 per cent inhibited TMV titer about the same as recorded for the stone fruit virus at that concentration. Malachite green at 0.001 M had little effect on TMV titer.

Guanazola at concentrations of 0.005, 0.01 and 0.05 per cent inhibits the virus. The percentage of inhibition is inconsistent (Table 6) and not so great as recorded for PLMV.

No infectivity tests with chemically treated TMV-infected tissue were made for comparison with spectrophotometric virus titer readings.

DISCUSSION.—Whereas valuable data are reported in the literature on chemotherapy of viruses, most of the work was done on excised plant parts and results are not necessarily expected to be similar to those obtained when chemicals are applied to a whole growing plant. The vacuum-infiltration procedure described has the limitation of a short period of exposure to the full concentration of chemicals tested; however, the procedure permits the use of whole plants and may permit better distribution of the chemicals within the plant. For screening purposes the method of treatment appeared to be sufficient, especially with a virus such as that used in this study which develops symptoms in cucumber 4–5 days after inoculation. In the case of TMV in tomato seedlings the treatment with thiouracil was sufficient to reduce spectrophotometric virus titer to less than 8 per cent of that in control plants when measured 7–10 days after treatment.

The percentage inhibition determined by the spectrophotometric virus titer was less than the percentage inhibition determined by infectivity in the case of the stone fruit virus in cucumber for chloramphenicol, thiouracil, and guanazola treatments. It is possible that this difference is due to inactive virus nucleic acid being included in the spectrophotometric analysis, which would not be indicated in the infectivity analysis. There is also the possibility that inactivation occurs during homogenation of the treated inoculum in the presence of residual chemotherapeutant. It is not the purpose of this paper to explain the therapeutic action, but to present preliminary results with 3 materials and a method of testing therapeutic agents with sufficient data to support the applicability of the procedure.

TREE FRUIT EXPERIMENT STATION
WENATCHEE, WASHINGTON

LITERATURE CITED

1. BEALE, HELEN PURDY, AND CAROLYN R. JONES. 1951. Virus diseases of tobacco mosaic and potato yellow dwarf not controlled by certain purified antibiotics. Contrib. Boyce Thompson Inst. **16**: 395-407.
2. COMMONER, B., AND F. L. MERCER. 1951. Inhibition of the biosynthesis of tobacco mosaic virus by thiouracil. Nature **168**: 113-114.
3. COMMONER, B., AND F. L. MERCER. 1952. The effect of thiouracil on the rate of tobacco mosaic virus biosynthesis. Arch. Biochem. and Biophys. **35**: 278-289.
4. COMMONER, B., P. NEWMARK, AND S. D. RODENBERG. 1952. An electrophoretic analysis of tobacco mosaic virus biosynthesis. Arch. Biochem. and Biophys. **37**: 15-36.
5. KIDDER, G. W., VIRGINIA C. DEWEY, R. E. PARKS, JR., AND G. L. WOODWIDE. 1949. Purine metabolism in Tetrahymena and its relation to malignant cells in mice. Science **109**: 511-514.
6. KIRKPATRICK, H. C., AND R. C. LINDNER. 1954. A rapid spectrophotometric assay for some plant viruses. Phytopathology **44**: 525-529.
7. KUTSKY, R. J., AND T. E. RAWLINS. 1950. Inhibition of virus multiplication by naphthalene acetic acid in tobacco tissue cultures as revealed by a spectrophotometric method. Jour. Bact. **60**: 763-766.
8. LEBEN, C., AND R. W. FULTON. 1952. Effect of certain antibiotics on lesion production by two plant viruses. Phytopathology **42**: 331-335.
9. LINDNER, R. C., H. C. KIRKPATRICK, AND T. E. WEEKS. 1952. Ultraviolet absorption spectra as a tool for diagnosing plant virus diseases. Science **115**: 496-499.
10. MATTHEWS, R. E. F. 1951. Effect of some substituted purines on the development of plant virus infections. Nature **167**: 892-893.
11. MATTHEWS, R. E. F. 1952. Effect of purines on the multiplication of plant viruses. Nature **169**: 500-501.

12. MEIKLEJOHN, G. 1949. Chemotherapy in viral and rickettsial disease. California Medicine **71**: 319-322.
13. MOORE, J. D., J. S. BOYLE, AND G. W. KEITT. 1948. Mechanical transmission of a virus disease to cucumber from sour cherry. Science **108**: 623-624.
14. NICKELL, L. G., PHYLLIS GREENFIELD, AND P. R. BURKHOLDER. 1950. Atypical growth of plants. III. Growth responses of virus tumors of Rumex to certain nucleic acid components and related compounds. Bot. Gaz. **112**: 42-52.
15. TAKAHASHI, W. N. 1948. The inhibition of virus increase by malachite green. Science **107**: 226.
16. STODDARD, E. M. 1942. Inactivating in vivo the virus of X-disease of peach by chemotherapy. Phytopathology **32**: 17.

STUDIES ON STONE-FRUIT VIRUSES IN CUCURBIT HOSTS. IV. SOME EFFECTS OF HYDROGEN ION CONCENTRATION AND DILUTION ON INFECTIVITY [1]

R. S. Willison and M. Weintraub

SUMMARY

The effects of hydrogen ion concentration on the infectivity of a number of stone-fruit virus isolates *in vitro* were studied in comparative aging tests at 0°C. conducted with aliquots of crude juice obtained from infected cucumber plants and diluted with water and buffer. The buffers used were 1 per cent dipotassium phosphate adjusted with citric acid to pH 8.5, 6.7, 5.7, and 4.7. Some isolates were more sensitive than others to pH changes. The influence of hydrogen ion concentration tended to become more pronounced at dilutions greater than 10^{-1}. The dilution tolerances of different isolates under the most favorable conditions ranged from 10^{-2} to more than 10^{-4}. Temporary inhibition of infectivity when present during aging occurred at the pH levels tested, but not always at the same time nor to the same extent.

Isolate Y.2 of the cherry yellows group could be separated into at least 2 components when aliquots of inoculum from abnormally dark green plants were diluted to 10^{-1}, one with water and the other with buffer at pH 5.7–6.0. The 2 components differed in induced symptoms, and in response to aging and thermal inactivation.

To pave the way for a study of the nature and characteristics of the group of stone-fruit virus entities transmissible to cucumber, it was found necessary to investigate the factors influencing infectivity and longevity of these entities in crude juice, before purification techniques could be perfected. Some effects of the environment of source and assay plants and of host constituents have already been discussed (3,6). The present paper reports some effects of hydrogen ion concentration on the behavior of several isolates at different dilution levels. Attention has been directed to date more towards ways and means of increasing or prolonging infectivity than towards determining end-points.

MATERIALS AND METHODS.—As in other papers of this series (3,4,5,6), each virus isolate is identified by a letter denoting the disease with which it is associated and a number signifying the source. For example, isolates G.1, T.2, N.1, and Y.2 were obtained from trees showing symptoms of green ring mottle, tatter leaf, necrotic ring spot, and cherry yellows respectively. Isolates G.1 and T.2 have each been separated into 2 components, designated A, which goes to cucurbit hosts, and B, which goes to cucumber, tobacco, and a number of other herbaceous hosts.

In the experiments reported herein, cucumber plants of the variety Windermoor Wonder were used for sources of inoculum and for assay. The system of infection rating employed in the assays has been fully described (5,6).

Inoculum was prepared by grinding diseased leaves in a mortar to which distilled water was added at the rate of 1 ml. per gm. of leaf. The homogenate was then divided into 5 aliquots further diluted with water or with 1 per cent K_2HPO_4 either alone or adjusted with citric acid to pH 6.7, 5.7, and 4.7 respectively, so that the final concentration of leaf material was 10^{-1}. Higher dilutions at 10^{-2}, 10^{-3}, and 10^{-4} were made in the usual way. The inocula were kept in a bath of ice-water and inoculated by a standard technique (6) to assay plants at intervals over a 48-hour period. The preparations diluted with buffer maintained the pH of the buffer except those diluted to 10^{-1} with phosphate alone (pH 8.5). In the latter preparations the pH stabilized at approximately 7.5. The hydrogen ion concentration of the water dilutions varied considerably from assay to assay, usually on the acid side of neutrality, but appeared to be constant for any one preparation, pH readings being virtually the same at the beginning and at the end of the 48-hour aging.

EXPERIMENTAL RESULTS.—As with other phases of the investigation (3,4,5,6), results varied with the

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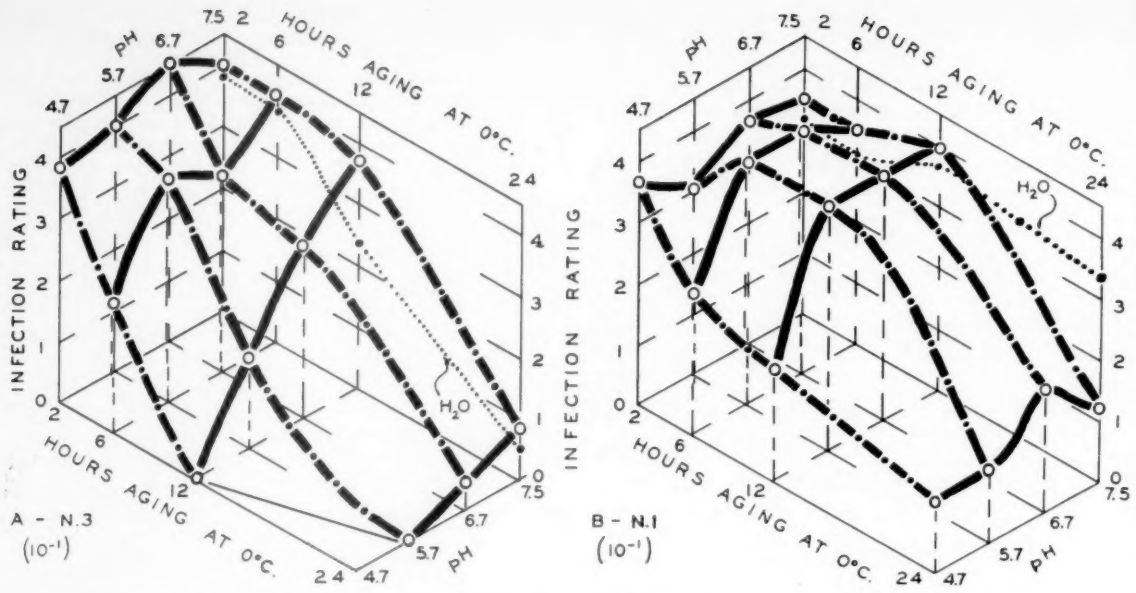

FIG. 1. Effect of pH on aging of inoculum at 0°C. and at a dilution of 10^{-1}. Source plants infected A) with isolate N.3 14 days before assay, Aug. 14, 1952; B) with isolate N. 1 10 days before assay on Aug. 25, 1952.

isolates and with the environmental conditions under which source and assay plants were grown. Since it is neither necessary nor desirable to cover in detail all the experiments performed, only representative results and a few special cases are dealt with.

The effect of pH on infectivity and aging.—With most isolates, infectivity decreased more or less gradually with time (Fig. 1). At dilutions of 10^{-1}, the infectivity of most isolates was not seriously affected during the first hour or so by pH adjustments within the range employed, except occasionally at pH 4.7. However, the effects of hydrogen ion concentration became more apparent as the inocula aged. Some isolates, e.g. N.3 and G.3, lost infectivity more rapidly with increasing acidity (Fig. 1,A), whereas others, e.g. N.1 and G.1B, appeared to be more stable over the range pH 7.5 to 5.7 (Figs. 1,B and 2,B respectively). With most isolates of the latter type, infectivity was markedly reduced after several hours at pH 4.7 (Fig. 1,B). At dilutions of 10^{-2} or higher, the effect of hydrogen ion concentration on longevity tended to become more pronounced.

Some isolates, notably G.1A, T.3 and Y.2, were sometimes but not always abnormal in their response to aging, infectivity rising, or fluctuating significantly with time (Fig. 2,A). Isolate N.2, unlike other isolates of the necrotic ringspot group, also showed fluctuations in infectivity during aging. If abnormal response to aging occurred, it was usually observed at all pH levels within the tolerance of a given isolate, though the fluctuations in infectivity did not necessarily occur at the same time in the aging process (Fig. 2,A). The temporary depressions of infectivity were in some cases less pronounced at or near the most favorable pH.

In Table 1, the several isolates under test are grouped according to the diluting medium in which they were most infective and most resistant to aging. In the first place, the groupings of isolates on this basis did not correspond to the groupings based on the symptom type induced on Prunus hosts. Similar wide variations in other characteristics among virus entities associated with a given Prunus virosis have been noted elsewhere (3,4,6). In the second place, some isolates, for example G.3 and N.1, varied in their response to pH or to the diluting medium at different levels of dilution. One isolate, Y.2, proved to be more than usually variable in different experiments. Thirdly, dilution with buffer was more favorable than dilution with water for the infectivity and longevity of all but 3 of the isolates (Table 1). The behavior of the water-diluted inocula, however, did not necessarily agree with that of similar inocula buffered at a comparable pH level. For example, in one experiment with isolate N.1, inocula were aged after centrifugation for 20 minutes at 3,000 G. The infection ratings after 12, 24, 36 and 48 hours aging were 3.8, 4.0, 3.6, 4.0 and 3.2, 2.2, 1.7 and 0.7 respectively for the water dilution at pH 5.2–5.6 and for the comparable aliquot buffered at pH 5.7. It would therefore appear that factors other than pH also influenced resistance to aging in inocula diluted with either water or buffer.

The effect of dilution.—As indicated above, the effects of hydrogen ion concentration on aging and on initial infectivity of a given isolate tended to become more pronounced as dilution increased. It was also apparent that dilution end-points would be more or less affected by pH as well as by environmental

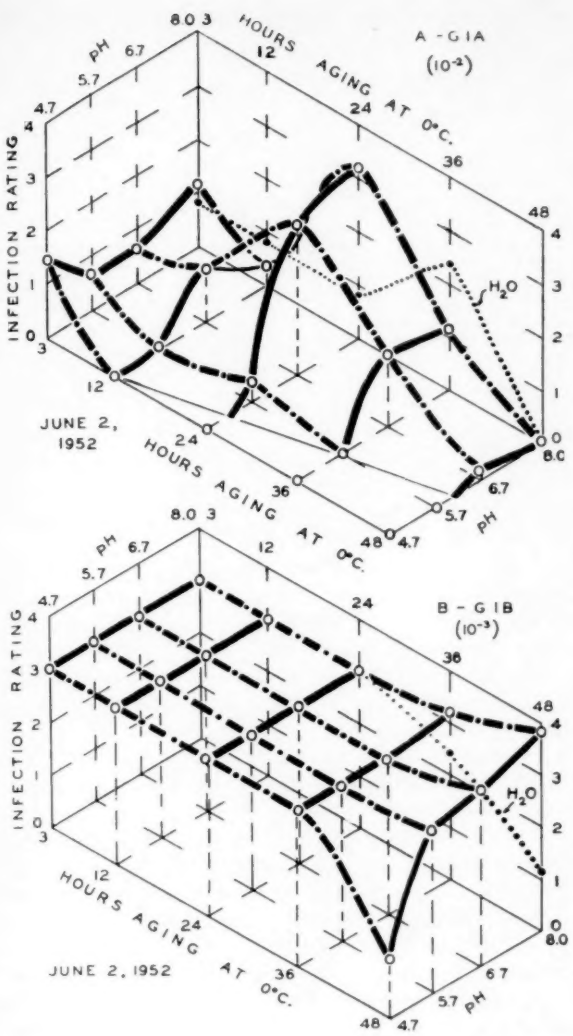

FIG. 2. Effect of pH on aging of inoculum at 0°C. Source plants infected 10 days before assay, June 2, 1952. A) isolate G.1A, at dilution 10^{-2}; B) isolate G.1B, at dilution 10^{-3}. Note: these 2 entities are components of a complex.

conditions before and after assay. The dilution end-points in Table 2, supplementing the information reported earlier (6), are those observed to date under the most favorable conditions of assay, including the diluting medium. Grouping of isolates according to dilution effects would appear to be as unpredictable as groupings according to other characteristics.

The effect of buffer on a complex.—An unexpected development arising from the adjustment of pH of inocula was the partial separation of 2 components from isolate Y.2 (cherry yellows group). Mention has been made elsewhere (3) of the very dark green pigment observed in some cucumber plants infected with isolate Y.2. This coloration became more prevalent as the autumn of 1951 advanced. It was at first thought that the production of the abnormally dark green pigment was a response to seasonal changes in environment. On Jan. 18, 1952, a new isolate of Y.2 was obtained from peach twigs which had been allowed to leaf out in the laboratory. The cucumber plants inoculated at this time and in subsequent transfers and assays were held under controlled conditions at 75–77°F. The plants infected in the course of the first 2 transfers of the new series were characteristically dwarfed but showed the very dark green color with comparatively little chlorotic marking. Inoculum prepared from such plants was low in infectivity. On Feb. 18, 1952, in an attempt to obtain more potent inoculum, 1 aliquot of homogenate was diluted to 10^{-1} with water and another with buffer at pH 6.0 for aging tests at 0°C. The plants inoculated with the water dilution developed the dark green symptoms (type A) as before, but those inoculated with the buffered aliquot were paler green and marked with abundant chlorotic spotting and ring patterns (type B symptoms). The occurrence of those 2 types of symptoms under the same conditions suggested that isolate Y.2 was a complex of at least 2 components, hereafter designated according to symptom type as components A and B.

Component A was evidently predominant in the inoculum diluted with water but was suppressed in the buffered inoculum to the point where component B could become recognizably established. The low infectivity of component B in the first separation in comparison with its infectivity in later aging tests (Table 3) supports the view that this component was at a relatively low concentration when first isolated.

To test the hypothesis that 2 components were involved, 2 aging tests were run with water dilutions at 10-day intervals. The assay plants of 1 experiment were used as the source plants for the next. Inocula of components A and B were prepared from plants selected for uniformity of symptom types A and B respectively. In each experiment, the 2 components retained their identity through transfer via water-diluted inocula (Table 3, aging tests I and II). In

TABLE 1.—*Classification of 11 isolates, according to optimum diluting medium*

Buffer at			
pH 7.0–8.0	pH 6.5–7.0	pH 5.7–6.5	Water
G.1A, G.1B	G.3 (10^{-1})[a]		G.3 (10^{-2})[a]
T.2B	T.3		T.2A
N.3	N.1 (10^{-2})[a]	N.2	N.1 (10^{-1})[a]
Y.2[b]	Y.2[b]	Y.2[b]	
		D.1	

[a] Number in bracket refers to dilution level.
[b] Experiments with Y.2 under different conditions of assay.

TABLE 2.—*Approximate dilution end-points of 11 isolates*

Dilution end-point	*Isolate*
$<10^{-2}$	T.3, D.1,
10^{-2}–10^{-3}	T.2A, N.3,
10^{-3}	G.1A, N.1, N.2, Y.2,
10^{-3}–10^{-4}	G.3
$>10^{-4}$	G.1B, T.2B

TABLE 3.—*Aging at 0°C. of isolate Y.2 in water and buffer at pH 5.7 to 6.0 and of components A and B in water (dilution 10^{-1}).*

Inoculum	Diluent	Infection ratings after aging at 0°C. 0 hr.	3–4 hr.	6–8 hr.	17 hr.	24 hr.	Sympt. Type predominant on assay plants
Separation I							
Original isolate	Water	*0.2*[a]	2.5	4.2	—	0	A
	Buffer	*0.2*	0.3	1.3	—	0	B
Aging test I							
Component A	Water	2.8	*1.7*	3.3	0.1	—	A
Component B	Water	3.4	3.2	3.2	2.7	—	B
Aging test II							
Component A	Water	2.5	—	*0.3*	2.0	0	A
Component B	Water	4.0	—	3.3	4.2	3.3	B
Separation II							
Component A	Water	2.0	*0.5*	0.6	1.6	0	A
	Buffer	3.1	3.0	0.9	1.6	0.1	B + A[b]

[a] Italics indicate initial or intermediate depressions in infectivity followed by a significant rise (Significant either by 't' test for difference between means (infection ratings), P= or <.05, or by coefficient of association (Q).
[b] Separation of B component not complete.

addition, component A was also differentiated from component B by its lower infection ratings, by its more rapid loss of infectivity and by its temporary inhibition during aging. As growing conditions during the pre-inoculation stage improved, the cucumber plants tended to become more susceptible to component B than to component A. The temporary depression and subsequent peak of infectivity for component A also shifted in time.

The first separation of the 2 components was not complete since some plants inocluated with component A showed type B symptoms in the second aging test. This was not surprising, as component A was essentially the original isolate carried through successive transfers in water dilutions, and in the course of time the assay plants became successively more susceptible to the B component. A second separation by means of water and buffer dilutions was made in April from plants inoculated with component A. The 2 aliquots of inoculum aged differentially as before but this time the respective infection ratings indicated a higher effective virus concentration for component B (separation II, Table 3). However, the separation of component B in the buffered inoculum was less clear cut than before.

For further confirmation of the occurrence of the components of Y.2, 3 thermal inactivation tests were made with each fraction, the first concurrently with the second aging test and the other 2 after the second separation (Table 4).

For the second and third tests, each component was diluted with water and with buffer. Inocula were prepared from leaves selected for the appropriate symptom type. It would appear to be more than coincidental that the B component when diluted with either water or buffer at 10^{-1} was characterized by a low level of infectivity after exposure to 35°C. for 10 minutes and high infectivity in controls and after exposure to 40° or 45°C. for 10 minutes, whereas these fluctuations in infectivity were not observed with component A. Furthermore, component A appeared to have a lower thermal coefficient (1,4) than component B.

DISCUSSION.—In the course of investigations with this group of fruit tree viruses (3, 4, 6), it has become increasingly evident that the determination of characteristics usually used for the identification of virus entities is here complicated by the occurrence in Prunus leaf homogenates of virus inactivators and in cucurbit homogenates of reversible inhibitors. Each isolate appeared to react in its own way to these inhibitors (3) and its reaction varied markedly with environmental changes and other factors. As with other factors, the effect of hydrogen ion concentration on the infectivity of an inoculum results from its action on the virus entity per se and also on the virus-inhibitor relation or complex. The effect of pH on the virus-inhibitor relationship, which could be expected to appear in tests with isolates that behaved abnormally during aging, was not consistent. In some cases, the amount of inhibition was appreciably reduced at favorable hydrogen ion concentrations and, in others, a change in pH was accompanied by a change in the timing of depression and recovery of infectivity.

One per cent dibasic potassium phosphate solution was chosen as the buffer because of its action in augmenting the infectivity of other virus preparations (2, 7). Also, in a preliminary test with isolate Y.2, inoculum diluted with that solution maintained a high level of infectivity for at least 24 hours longer than that diluted with water. In this experiment, the inocula were prepared from infected plants that were abnormally dark green. However, evidence for the "phosphate effect" as defined by Yarwood (7) was not obtained in the later experiments, since at dilutions higher than 10^{-1}, no outstanding increases in infection

TABLE 4.—*Thermal inactivation of components A and B, of isolate Y.2 (dilution 10^{-1})*

Component	Diluent	Infection ratings after 10 minutes at							
		Control	35°C.	40°C.	45°C.	50°C.	55°C.	60°C.	65°C.
Test I									
A	water	1.7	0	0	0.3	0	0	0	0
B	water	3.7	*2.0*[a]	3.5	3.9	2.4	2.1	0	0
Tests I and II (results combined)									
A	water	2.5	2.7	2.3	1.8	0.3	0.4	0.2	0
B	water	2.9	*1.6*	2.2	2.7	0.4	0.2	0	0
A	buffer	2.9	2.0	1.4	1.5	0.1	0.3	0	0
B	(pH 5.7)	3.6	*2.4*	3.2	1.6	0.5	0.5	0	0

[a] Italics indicate infectivity at 35°C. exposure significantly lower than at 40° or 45°C. ($P < .05$ for significant difference between means (infection ratings)).

rating or longevity were gained by substituting 1 per cent phosphate for water as a diluent. In fact, with some isolates, dilution with buffer actually shortened longevity. Yarwood (7) also found that the phosphate effect did not occur when cucumber plants were inoculated with tobacco-necrosis virus.

The partial separation of 2 components present in isolate Y.2 by means of water and buffer dilutions provides additional evidence that different virus entities associated with stone-fruit viroses respond differentially to changes in either the hydrogen ion concentration or the composition of the diluting medium. The evidence for the occurrence of 2 components in isolate Y.2 is 3-fold: 1) differences in symptom expression not only occurred under the same conditions of assay but also persisted through transfers with inocula prepared with the same diluent; 2) differences in the aging characteristics of the 2 components in the same medium of dilution were reproducible under comparable conditions of assay; 3) differences in behavior of the 2 components during thermal inactivation tests persisted with both water and buffer dilutions on at least 2 occasions under similar assay conditions.

On more complete analysis of the data, the hypothesis that the inhibitor associated with abnormally dark green coloration was inactivated at 40°–45°C. (3) has become untenable. That inhibitor may have been responsible for the low thermal coefficient of component A of isolate Y.2 or it may have been inactivated at 35°C. It also appeared to have become inactivated before the virus in aging tests at 0°C. On the other hand, the inhibitor affecting component B gave no evidence of activity during aging at 0°C. but appeared to be sensitive to thermal effects. Inhibition was increased by a 10-minute exposure to 35°C. but was not observed after treatment at 40°–45°C.

Variation in results obtained with isolate Y.2 (Table 1) can be explained, in some degree, on the grounds that the isolate is a complex of at least 2 components, which may vary from time to time in their relative concentration in host and inoculum.

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LITERATURE CITED

1. BAWDEN, F. C. 1950. Plant viruses and virus diseases. Ed. 3. Chronicia Botanica Co., Waltham, Mass. 335 p.
2. THORNBERRY, H. H. 1935. Effect of phosphate buffers on infectivity of tobacco-mosaic virus. Phytopathology **25**: 618-627.
3. WEINTRAUB, M., AND R. S. WILLISON. 1953. Studies on stone-fruit viruses in cucurbit hosts. III. The effect of cucurbit extracts on infectivity. Phytopathology **43**: 328-332.
4. WEINTRAUB, M., AND R. S. WILLISON. 1954. Studies on stone-fruit viruses in cucurbit hosts. V. Thermal inactivation of some isolates. Phytopathology **44**: 538-542.
5. WILLISON, R. S., AND M. WEINTRAUB. 1953. Studies on stone-fruit viruses in cucurbit hosts. I. A method of evaluating the infectivity of infectious juice. Phytopathology **43**: 175-177.
6. WILLISON, R. S., AND M. WEINTRAUB. 1953. Studies on stone-fruit viruses in cucurbit hosts. II. Some factors affecting the aging of inoculum in vitro. Phytopathology **43**: 324-328.
7. YARWOOD, C. E. 1952. The phosphate effect in plant virus inoculations. Phytopathology **42**: 137-143.

STUDIES ON STONE-FRUIT VIRUSES IN CUCURBIT HOSTS. V. THERMAL INACTIVATION OF SOME ISOLATES [1]

M. Weintraub and R. S. Willison

SUMMARY

Thermal inactivations of 13 stone-fruit virus isolates were studied over a period of time, and under various environmental conditions for growth of the source and assay plants. It was not found possible to assign with certainty any definite thermal inactivation point to each of the isolates. This was due to the variability of the end-point, which shifted by as much as 15°C. with varying conditions. One of these environmental conditions was found to be the air-temperature at which the source plants were grown.

Most of the isolates yielded thermal inactivation curves indicating either a large or a small temperature coefficient. However, this criterion was also not consistent, since over a period of time the character of the inactivation curve for several isolates appeared to change from that for a large to a small thermal coefficient.

One of the purposes of these studies is the differentiation, as far as possible, of those properties that are characteristic of stone-fruit virus entities *per se*, and those that are conditioned by the host, the host-virus combination, and environment. This paper accordingly reports results of exposing crude infective sap to various temperatures. Since the crude juice of diseased stone-fruit leaves has a very short period of virus activity (5), all experiments were conducted with sap derived from infected cucumber plants.

MATERIALS AND METHODS.—The virus entities used in this study, described previously (3, 4, 5), were transmitted from peach leaves to young cucumber plants by mechanical inoculation. Each isolate is identified by a letter indicating the group according to the symptom type observed on the Prunus hosts: Y, cherry yellows; N, necrotic ring spot; D, prune-dwarf; G, green-ring mottle; T, tatter-leaf. The number appended to each letter identifies the original tree source of a particular isolation.

Two of the isolates, G.1 and T.2, were each separated into 2 distinct components, designated respectively as G.1.A and G.1.B, T.2.A and T.2.B. Other characteristics and properties of these components will be described in a later paper.

The procedure for thermal inactivation tests was standardized as far as possible. Tests were made with crude, unstrained juice of infected cucumber leaves, the juice being diluted to 10^{-1} in distilled water, and divided into 8 aliquots. Except for the 10-minute exposure at the desired temperature, the aliquots were kept in an ice-water bath until the assay inoculations were made. The juice was at pH 5.6–5.8, and no change in pH was observed in the time required to run an inactivation test. In each run, each of 7 aliquots was exposed for 10 minutes to temperatures from 35°C. to 65°C., at 5 degree intervals while the eighth aliquot was used as an unexposed control. The exposure and assay inoculation for the 65°C. aliquot was done first, that for 35°C. last, and the control was assayed halfway through the test. The inactivation tests for each isolate were replicated at least twice, and for some as many as 15 times.

The method for assaying infectivity (4) is a numerical assessment of the average severity of the disease induced in a group of 5 or 10 cucumber plants inoculated at 1 time with a given virus preparation. A rating of 5 indicates that all plants in the inoculated group showed necrosis of the growing point; 4, that growth in all plants ceased without necrosis; 3, that all plants showed systemic symptoms, but not severe dwarfing; and 0, that no plants became infected. Intermediate values indicate that, in some of the inoculated plants, the disease did not reach the maximum severity characteristic of the isolate under the conditions in which it was assayed.

EXPERIMENTAL RESULTS. — *Comparison of isolates from different groups.*—The manner in which the isolates lost their infectivity with successive increases in temperature divided them into 2 distinct groups (Fig. 1A). One group, which included isolates N.1, D.1, and G.1.A., began with a high infection rating (4.5–5.0), and this rating was maintained at a high level over a range of approximately 20°C., temperatures between 35°C. and 50–55°C., causing relatively little or no loss of infectivity. The critical temperature, however, appeared to be close to 55°C., and small increases in temperature beyond this point resulted in large increases in inactivation of the viruses, all isolates being completely inactivated at 65°C. The only exception to this was isolate N.1, the critical temperature point of which was somewhat lower (50°C.); it was inactivated completely at the correspondingly lower temperature of 60°C. Isolates G.2 and Y.2 were tested together with this group, but since they produced essentially the same results as those obtained with isolate G.1.A., they were omitted from Fig. 1A.

The second group, including isolates G.3 and T.5, produced inactivation curves that were radically different in several respects (Fig. 1A). The infection ratings of the unheated controls were relatively low (0.8 and 1.5 respectively), but became higher with increase in temperature. Exposures to temperatures in excess of 40-45°C. resulted in a gradual decline in infectiv-

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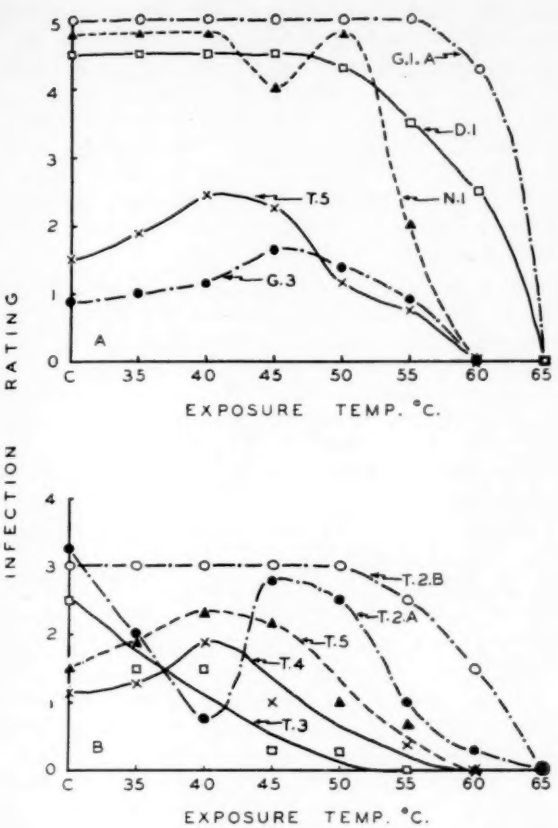

FIG. 1. Infection ratings of virus preparations after exposure for 10 minutes to various temperatures. C = unexposed control. A) Comparison of 5 isolates (G.1.A, D.1, N.1, T.5, and G.3) from 4 different symptom groups. B) Comparison of 5 isolates from the tatter-leaf symptom group.

ity, with complete inactivation at 60°C. The significant rise in infectivity with increase in temperature from 35°C. to 45°C. is a phenomenon that has been observed previously with other isolates, and is reproducible. It is attributed to the presence of inhibitors in the cucumber leaf tissue, which are presumably inactivated at these temperatures, resulting in an increase of infective virus (3).

The third point of difference between the 2 groups was that of the form and slope of the inactivation curve. The first group reacted most sensitively to small increases in temperature only beyond a critical point, and might be said to have a large thermal coefficient. The second group had a relatively wide range of inactivation temperatures, and was characterized by a small thermal coefficient.

It is evident that this grouping by the rate of thermal inactivation cuts across the grouping of isolates by symptom expression in the Prunus hosts. Thus of the 3 G isolates used in this test, G.1.A., and G.2 fell into the group possessing a critical thermal point, followed by a precipitous inactivation, whereas isolate G.3 was in the second group, with a wide range of inactivation temperatures.

Comparison of different isolates within one group.—Five separate isolates of the tatter-leaf symptom group were compared as to the manner of their thermal inactivation (Fig. 1B). It was clearly evident that the isolates differed in varying degrees in successive loss of infectivity. Isolate T.2.B, an entity very similar in all properties to isolate G.1.B, produced a thermal inactivation curve distinctly of the large thermal coefficient type. Isolate T.3 was almost certainly characterized by a small thermal coefficient. Of the other 3 isolates, T.4 and T.5 were of the type discussed previously. Their infection ratings were first depressed by the inhibitors present in the cucurbit tissues, but as these inhibitors were (presumably) inactivated at 35–40°C., the infection ratings proceeded to increase, and then slowly to decrease over a 20°C. range. Thus, these 2 isolates could be tentatively included as virus entities with a small temperature coefficient.

Isolate T.2.A appeared to be rather more complex in its reactions to increases of exposure temperature. Exposures at 35°C. and 40°C. each produced a marked decrease in the infection rating, but exposure at 45°C. raised the infection rating to a point almost equal to that of the untreated control. Successively higher temperatures then decreased the infection ratings to complete inactivation at 65°C., although the low rating produced at 40°C. was not reached again until a temperature between 55–60°C. The decline in infection rating above 45°C. was relatively proportional to the successive increases in temperature, but the reasons for the rapid decrease in rating between ± 0°C. and 40°C., followed by an equally rapid increase in rating between 40°C. and 45°C. are not completely understood. In part these reactions may be attributed to a greater sensitivity of isolate T.2.A to the natural inhibitors than was evidenced by isolates T.4 and T.5. It has been observed that cucumbers infected with at least 1 component of Y isolates, namely component Y.2, produced under certain conditions a thermal inactivation pattern almost identical with that of T.2.A. Because of the complex type of inactivation curve produced by isolates T.2.A and Y.2, it becomes difficult to include these isolates definitely in either of the 2 thermal coefficient groups set up.

Comparison of 2 components from the same isolate.—The 2 components of isolate G.1, comprising G.1.A. and G.1.B., were compared in their rates of thermal inactivation. The tests reported here were done on 2 separate occasions, July 21, and October 22, with comparable material. For the test of July 21, the 2 components fell into 2 groups, G.1.B. having a large thermal coefficient, while G.1.A. with its wider range of temperatures over which infectivity was lost, had a small thermal coefficient (Fig. 2A).

However, when G.1.A. was tested on October 22, it produced a curve attributable to a virus with a large thermal coefficient. It would thus appear that the form of the inactivation curve for isolate G.1.A. changed with the different conditions under which the experiment was conducted. Isolate G.1.B. on the other hand did not show this change with varying conditions,

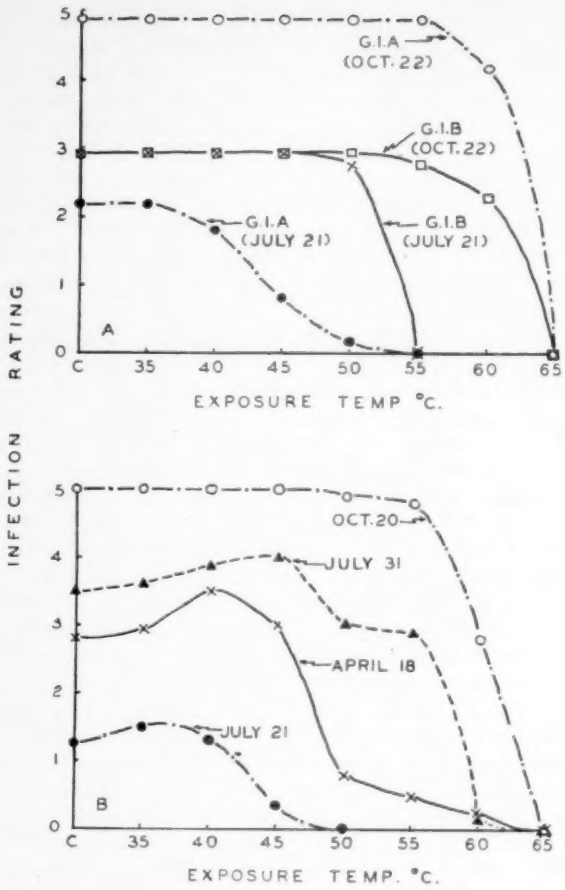

FIG. 2. Infection ratings of virus preparations after exposure for 10 minutes to various temperatures. C = unexposed control. A) Comparison of 2 components derived from one isolate, tested on 2 separate occasions. B) Effects of various environmental conditions on isolate Y.2.

and the inactivation tests at different times produced essentially the same type of curve, evidencing a large thermal coefficient (Fig. 2A). In this case, however, different conditions in the environment shifted the thermal inactivation point from complete inactivation at 55°C. in July to 65°C. in October. Thus, the different components of single isolates reacted to environmental conditions either by a change in the form of the thermal inactivation curve, or by a shift in the thermal inactivation point.

Effects of environment on one isolate.—In addition to the effects that different environmental conditions exerted on 2 distinct components of 1 isolate, certain effects were observed on single isolates as their thermal inactivation was studied over a period of time. A comparison of the data for isolate Y.2 (Fig. 2B) for the tests of July 21 and July 31 shows strikingly the effect of at least 1 environmental factor, namely the air temperature at which the source and assay plants were grown. The outdoor air temperatures on July 21 were very high, ranging in the high 80's, and those of the week before and after this date were uniformly high. On the other hand, beginning with July 29, outdoor air temperatures steadily declined to the low 70's, in the first week of August. Under these conditions, the range of greenhouse temperatures were, of course, considerably higher than outdoor temperatures, both day and night. It is evident that the point of complete thermal inactivation of the Y.2 preparations was reduced from 65°C. in May, October, and July 31, to 50°C. on July 21.

Although the varying conditions during the tests of October 20, April 18, and July 31 did not shift the point of complete thermal inactivation, they resulted in a lowering of the initial infection rating, as well as changing the form of the inactivation curve so that in the tests of April 18 and July 31, the critical temperature at which infectivity began to decline sharply, was neither so well defined nor so high as that obtained on October 20.

Similarly, isolate N.3 reacted to tests on July 21 and July 31 in much the same way as did isolate Y.2 (Table 1). Not only was the general form of the inactivation curve changed, but again as with isolate Y.2, the point of complete inactivation was shifted from 50°C. (July 21) to 60°C. (July 31).

On the other hand, isolate Y.3 showed less sensitivity than either Y.2 or N.3, to the different conditions imposed during tests on July 21 and 31 (Table 1). The general form of the inactivation curve was maintained on both days, although the infection ratings throughout the test on July 21 were somewhat lower than those for July 31. The temperature required for complete inactivation in this case shifted only 5°C., from 50°C. (July 21) to 55°C. (July 31). Thermal inactivation tests for isolate Y.3 could not be carried out in September or October, since the virulence of this isolate increased to such an extent that it was not possible to harvest sufficient diseased foliage for the tests.

In order to determine whether the thermal inactivation curve and end point could be changed by artificial control of temperature, cucumber plants were inoculated with isolate T.2.B., and grown at 75, 80, and 85°F. until maximum symptom expression was observed. This material was subjected to thermal inactivation tests, and the assay plants were then grown at 75°F. Even this isolate, which is remarkably stable as compared to some of the other stone-fruit virus isolates, showed some effect of temperature on its thermal inactivation (Table 1). The primary effect was on the end-point of inactivation, the preparation from the plants grown at 75°F. being completely inactivated at 55–60°C., that from plants grown at 80°F. being inactivated at 50°C., and that from plants grown at 85°F. being inactivated at 45–50°C.

DISCUSSION.—The characterization of virus entities is based upon a number of criteria, beyond that of symptomatology, one of these being thermal inactivation. Although it was at first hoped that study of the end-points or rates of thermal inactivation might assist to some degree in differentiating the isolates obtained

TABLE 1.—*Effect of environmental conditions on thermal inactivation of stone-fruit virus isolates of various groups*

Isolate	Conditions	Infection rating of control	Infection rating of preparation exposed for 10 min. at 35°C.	40°C.	45°C.	50°C.	55°C.	60°C.	65°C.
Y.3	July 21, 1952	3.4	3.5	3.6	2.7	0	0	0	0
	July 31, 1952	4.5	3.9	4.0	4.6	2.5	0	0	0
N.3	July 21, 1952	1.1	2.3	1.8	0.8	0	0	0	0
	July 31, 1952	4.4	4.2	3.9	4.1	3.8	3.0	0	0
T.2.B.	July 21, 1952	3.0	3.0	3.0	3.0	2.8	0.5	0	0
	July 31, 1952	3.0	3.0	3.0	3.0	2.8	0	0	0
	May 23, 1952 controlled at:	3.0	3.0	3.0	3.0	3.0	2.4	1.5	0.2
	75°F.	3.0	3.0	3.0	2.5	1.2	0.2	0	0
	80°F.	3.0	3.0	2.8	1.2	0	0	0	0
	85°F.	2.4	2.4	1.8	0.5	0	0	0	0

from the Prunus hosts, this hope was not realized. The primary reason for this failure may probably be attributed to the variability of both the quantitative manner in which the virus is inactivated with successive increases of heat exposure, and the temperature at which complete inactivation is attained. This variability would appear to be a function at least of environmental conditions.

It is accordingly not feasible to state that any given isolate is characterized by a particular thermal inactivation point with the expectation that these results can be duplicated with certainty. Although the literature on stone-fruit viruses contains the statement that "The cherry virus had a thermal inactivation point of 52°C. for 10 minutes," the authors referring to a virus transmitted mechanically to cucumber from sour cherry showing necrotic ring spot symptoms (2), the results of the study reported here can neither corroborate nor gainsay this statement. It has already been noted with the necrotic ring spot isolate tested, N.3, that different environmental conditions may effect a spread of 10–15°C. in the thermal inactivation point (Table 1). A 15°C. spread is also seen with isolate Y.2 over a period of time (Fig. 2B). Similarly isolate G.1.B had a thermal inactivation point of 55°C. on July 21, 65°C. on October 22, (Fig. 2A), and survived 10 minutes at 65°C. in a test on May 23 (not figured). The sensitivity of the criterion of thermal inactivation to environmental conditions is thus made apparent.

In seeking a possible explanation for these significant changes in the inactivation points with different tests, a clue is provided by a study of the factors affecting the aging of stone-fruit viruses *in vitro*. It has been shown (5) that inoculum derived from cucumber plants grown at 75°F. has a higher level of both potential and effective virus concentration than that derived from plants grown at 85°F. It follows, therefore, that those preparations tested for thermal inactivation endpoints after a period of high air temperatures (e.g. July 21) contained less infective virus than preparations tested after a period of lower air temperatures (e.g. July 31). It might then be expected that the preparation with less infective virus would reach the minimum number of infective units remaining in the inoculum, at a lower exposure temperature than would the preparation with more initial infective virus.

The relative constancy of environmental conditions would also appear to exert some effect on thermal inactivation patterns. A comparison of the results obtained with isolate T.2.B from plants grown in the greenhouse and assayed on July 21, and from plants grown at 75°F. (Table 1), shows that despite the theoretically better temperature conditions of the latter for virus multiplication, the plants grown in the greenhouse produced the higher infection ratings after exposures to 45, 50, and 55°C. It is, however, difficult to explain these results on the basis of any one environmental factor. The greenhouse set was subjected to widely fluctuating temperature, light and possibly humidity, whereas the "controlled" set was grown at a constant temperature, day and night, 12 hours of illumination of 1200 foot-candles, and a relatively constant humidity. It is therefore possible that fluctuations in one or all of these conditions in the greenhouse may give rise to relatively higher infection ratings than do constant conditions.

These suggestions can account only partially for the serious discrepancies in thermal inactivation endpoints. It must be recalled that all tests were carried out with crude juice of infected plants, so that the condition of the host may play some part in effecting these discrepancies.

The thermal inactivation curves obtained with the various virus entities, as shown in the figures, are also of interest. Some of these curves are undoubtedly of a type indicating a virus entity with a large thermal coefficient. The form of the curves obtained from those isolates characterized by a small thermal coefficient are, in some cases, obscured by the effects of the natural inhibitors present in the host tissue (cf. Fig. 1A, T.5 and G.3 with Fig. 1B, T.3). However, if these obscured curves are extrapolated backwards to eliminate the superimposed inhibitor effect, it becomes evident that the form of the curves are characteristic of a small thermal coefficient, the infectivity falling proportionally with increase in temperature, over a wide range of temperatures, and a critical thermal point beyond which inactivation is precipitous, being absent.

Although Bawden implies (1) that the magnitude of the temperature coefficient is characteristic of the par-

ticular virus, there was some evidence in the present study that this is not necessarily the case. The best example is afforded by isolate G.1.A (Fig. 2A), the thermal inactivation curve of which changed radically when tested in October and in July. In the former test, the thermal coefficient for inactivation of the preparation was large, whereas in the latter test, it was small. This change, coupled with the fact that the thermal inactivation end-point for the isolate was discrepant by 10°C. suggests that the physiological condition of the host plant may govern both of these virus characteristics. An alternative possibility, or additional factor, may be the composition of the virus isolates. Since isolates G.1.A and Y.2 (6) consisted of more than 1 entity, it is, therefore, possible that over a period of time the several components of such an isolate may in turn impose their separate characteristics on any particular criterion being studied. This would be especially true if there is a flux in the relative concentration of the component entities over a period of time. Until these isolates can be purified, and freed from the host constituents, it is not possible to judge the extent to which such constituents contributed to these discrepancies.

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LITERATURE CITED

1. BAWDEN, F. C. 1950. Plant viruses and virus diseases. Ed. 3. Chronica Botanica Co., Waltham, Mass. 335 p.
2. BOYLE, J. S., J. D. MOORE, AND G. W. KEITT. 1949. Comparative studies of a virus disease of cucumber transmitted mechanically from sour cherry and several cucumber virus diseases. (Abs.) Phytopathology **39**: 3.
3. WEINTRAUB, M., AND R. S. WILLISON. 1953. Studies on stone-fruit viruses in cucurbit hosts. III. The effect of cucurbit extracts on infectivity. Phytopathology **43**: 328-332.
4. WILLISON, R. S., AND M. WEINTRAUB. 1953. Studies on stone-fruit viruses in cucurbit hosts. I. A method of evaluating the infectivity of infectious juice. Phytopathology **43**: 175-177.
5. WILLISON, R. S., AND M. WEINTRAUB. 1953. Studies on stone-fruit viruses in cucurbit hosts. II. Some factors affecting the aging of inoculum in vitro. Phytopathology **43**: 324-328.
6. WILLISON, R. S., AND M. WEINTRAUB. Studies on stone-fruit viruses in cucurbit hosts. IV. Some effects of hydrogen-ion concentration and dilution on infectivity. Phytopathology **44**: 533-537.

MEADOW NEMATODES ASSOCIATED WITH FAILURE OF SPRING-SOWN ALFALFA[1]

Richard A. Chapman

SUMMARY

Meadow nematodes, *Pratylenchus* sp., were found in large numbers in roots of alfalfa that had been sown in winter wheat in the spring. As many as 104,500 nematodes and eggs per gm. of fresh roots were found in 2-months-old seedlings. Root damage was extensive and severe, and stand was severely reduced. After summer fallow, fall-sown alfalfa plants of similar size in the same plot had approximately 1/1000 as many *Pratylenchus* as spring-sown plants. Root injury was slight and stand was good.

Failure of spring-sown alfalfa (*Medicago sativa* L.), especially when it is planted in a small grain, occurs frequently in Kentucky. During the past 2 decades these failures have resulted in a switch from the custom of planting alfalfa in the spring to the present practice of sowing this crop in the fall, usually after a period of summer fallow. Several times a field of winter wheat on the Experiment Station farm was sown with alfalfa in the spring. Each time the stand was so poor that the field was plowed and left fallow during the summer. Limited observations of spring alfalfa from this field showed that it had poorly developed, discolored roots. Good stands of alfalfa were obtained when planted in the fall.

The appearance of these roots was reminiscent of that of diseased clover roots that had been studied in detail by Fergus and Valleau.[2] In the case of the clover roots, several species of *Fusarium* were indicated as possible causes of the injury to the roots, but conclusive proof was not obtained. The descriptions and illustrations of the clover roots are typical for roots that have suffered injury by nematodes. Nematodes were not taken into account in this early study. About 20 years later, studies made by Valleau and

[1] Accepted for publication April 26, 1954.
The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published with the approval of the Director.

[2] Fergus, E. N. and W. D. Valleau. 1926. A study of red clover failure in Kentucky. Ky. Agr. Exp. Sta. Res. Bul. 259.

Johnson[3] indicated that meadow nematodes, *Pratylenchus* sp., were primarily responsible for the type of injury observed in the clover roots. The present study was undertaken to determine whether nematodes are a factor in failure of spring-sown alfalfa.

METHODS.—A variety test planting of alfalfa sown in winter wheat in the spring of 1953 was chosen for study.[4] Corn preceded the wheat.

Wheat samples were dug the day (March 20) alfalfa was sown in the spring in order to get an idea of the kind and number of nematodes in the plot just prior to development of alfalfa. The spring-sown alfalfa was sampled 3 times. Such a poor stand resulted from this alfalfa planting that the plot was plowed after the third sampling and left fallow during the summer, after which a fall planting was made. The fallow soil was sampled 8 times before germination of the fall-sown seed, and fall-sown alfalfa was sampled 4 times. Soil containing alfalfa and wheat was dug with a trowel from scattered locations in the 230 by 120 ft. plot. The samples varied in weight from 200–500 gm., and contained from 1–20 plants. All samples were wrapped in waxed paper and stored in a cold room until they could be processed. No sample was stored for more than 10 days.

The samples were processed by soaking them in water and carefully removing the plants in order to recover as much of the root system as possible. Roots were washed, blotted in a towel, and weighed. Roots of wheat plants were comminuted in a Waring blendor for 20 seconds and the comminuted material placed in Baermann funnels. Alfalfa roots were placed in water in Petri dishes or Baermann funnels.

Nematodes were collected from soil by repeatedly suspending soil in water and pouring the suspended material through U. S. 40 and U. S. 200 screens. Material collected on the screens was washed into Baermann funnels.

Numbers and kinds of nematodes in soil and in comminuted roots were determined from samples taken from the Baermann funnels 48 to 96 hours after the material was placed in them. Nematodes from non-comminuted roots were examined periodically over periods of 2–4 weeks. With the aid of a stereoscopic microscope all nematodes from 20 per cent of the surface area of dishes containing the samples were picked out by means of micropipettes and transferred to depression slides. Counts and identifications were made by examination through a compound microscope.

All references to numbers of nematodes are in terms of the number of nematodes per gm. of fresh root and the number of nematodes per kg. of soil.

RESULTS.—*Spring alfalfa.*—Preliminary examination of the nematodes associated with wheat in this plot indicated that meadow nematodes, *Pratylenchus* sp.,[5] predominated in number so far as potentially pathogenic kinds were concerned. They were far more numerous than others in root samples and were often more numerous in soil samples. Their occurrence in the soil was probably the result of roots breaking off plants during washing. Other possibly phytopathogenic nematodes found were *Helicotylenchus* sp., *Hoplolaimus* sp., *Xiphinema* sp., *Tylenchus* sp., *Dity-lenchus* sp., *Aphelenchus* sp., and *Aphelenchoides* sp. None of these was numerous in the wheat soil samples, and although some of them were occasionally found to be more numerous in some of the subsequent alfalfa samples, the increase was inconsistent and slight when compared with the consistent and large increase in the number of *Pratylenchus*.

The number of *Pratylenchus* found in the various samples is in Table 1. Forty-eight samples of wheat were examined. The counts from wheat roots represent only those nematodes released by comminution. Other wheat-root samples examined in connection with other work have yielded much higher numbers when examined periodically over a period of weeks than when examined after 1 or 2 days' soaking. This is probably because eggs hatched during the longer time. In the procedure used with these wheat samples any eggs that might have been in the roots escaped detection.

The variability in the number of *Pratylenchus* in these samples indicates that their concentration varied from place to place in the plot. However, there was no large area free from them or even having very low concentration.

Twenty-two samples of alfalfa were dug 2 months (May 20) after seeding. The wheat was in head at this time and formed a dense shade over the alfalfa. The alfalfa plants were quite small, the larger ones being approximately 1 in. tall with 1 or 2 true leaves. Their roots weighed 0.01 to 0.06 gm. per plant. The plants were light green and had yellow cotyledons and the roots were light brown.

As the data in Table 1 show, as many as 104,500 *Pratylenchus* were found per gm. of fresh alfalfa roots. Larvae predominated in most counts after the first 48 hours soaking, and after 7–10 days almost all nematodes were larvae, indicating that eggs hatched during the observation period. These counts represent the total nematode potential, adults, larvae, and eggs, present in the roots at the time of sampling.

As in the case of wheat, there was considerable variation from sample to sample, but in all the number of nematodes was large. The number of *Pratylenchus* recovered from alfalfa soil was approximately the same as from wheat soil.

A second set of 22 alfalfa samples was dug 1 week (May 27) after the first. Size of the plants is indi-

[3] Valleau, W. D. and E. M. Johnson. 1947. The relation of meadow nematodes to brown root rot of tobacco. Phytopathology 37: 838-841.

[4] The use of the planting was made available through the cooperation of Lawrence Henson of the Agronomy Department of the Kentucky Agricultural Experiment Station.

[5] The population of *Pratylenchus* encountered in this study was quite varied. Because it is not known whether the population is a mixture of species, or is made up of 1 or more variable species, it seems best to make no specific designation at this time.

TABLE 1.—*Number of Pratylenchus in roots of wheat and alfalfa and in wheat, alfalfa, and fallow soil*

Date and kind of sample	Number of samples	*Pratylenchus* per kg. of soil				*Pratylenchus* per gm. of fresh root			
		Samples with *Pratylenchus*	Highest concentration	Lowest concentration	Mean	Samples with *Pratylenchus*	Highest concentration	Lowest concentration	Mean
		Per cent				Per cent			
Wheat 3/20/53	48	71	982	0	131	77	50	0	13
Alfalfa 5/20	22	89	1,520	0	363	100	104,500	2,050	27,193
Alfalfa 5/27	22	91	1,019	0	210	100	42,391	1,111	12,188
Alfalfa 7/2	22	....	.....	....	....	100	1,850	16	556
Fallow soil 7/23	2	....	177	66	122	....	.....	....	....
Fallow soil 8/5	2	....	110	73	92	....	.....	....	....
Fallow soil 8/11	2	....	309	73	191	....	.....	....	....
Fallow soil 8/17	2	....	73	66	70	....	.....	....	....
Fallow soil 8/26	2	....	309	213	261	....	.....	....	....
Fallow soil 9/2	2	....	471	294	383	....	.....	....	....
Alfalfa 9/11	22	....	.....	....	....	14	250	0	25
Alfalfa 9/24	22	....	.....	....	....	27	243	0	25
Alfalfa 10/13	22	....	.....	....	....	68	227	0	49
Alfalfa 1/18/54	44	....	.....	....	....	100	1,840	67	440

cated by the weights of fresh roots, which varied from 0.0025 to 0.147 gm. per plant. Fewer *Pratylenchus* were found in these samples than in the first set, but the number was still large.

A third set of 22 alfalfa samples was dug 6 weeks later (July 2). The wheat had been cut by this time and the surviving alfalfa plants had made some growth. The larger ones were 2–3 in. tall and most of them had developed taproots with sparse, poorly developed, brown lateral roots. The fresh-root weights ranged from 0.03 to 0.26 gm. per plant. The stand of alfalfa was so poor at this time that the plot could not be used for a variety test, and it was plowed up shortly after this last set of samples was dug.

The number of *Pratylenchus* found in these plants was considerably less than that in the previous ones. However, the nematodes were probably concentrated in the few surviving lateral roots that made up only a small fraction of the total root weight. Consequently, the concentration of nematodes in the invaded parts of the roots was probably of the same order of magnitude as in the previous samples.

Fallow.—The plot was plowed about July 10. Excellent weed control was obtained by disking several times during the summer. Samples of the top 4–5 in. of soil were dug 8 times at approximately weekly intervals during the period between destruction of the spring planting and germination of the fall-sown seed. Trowel samples were dug every 20 ft. in 2 lines across the plot, 1 in the north half and 1 in the south half. The soil dug from each half was pooled and mixed. A 680 gm. aliquot was taken from each pooled sample and processed to determine its nematode population. The remainder was distributed into four 6-in. pots and planted with Atlantic alfalfa. The pots were set in a greenhouse, and 2 plants were permitted to grow in each pot. With 1 exception the alfalfa in the pots was harvested after 57 days, and the nematode population in the roots determined. One planting failed to grow well and some of the pots had to be resown. Thus, 2 groups of plants of different ages resulted.

The number of *Pratylenchus* in the field soil during the fallow period is in Table 1. Several varieties of alfalfa were planted on August 18 but, because of dryness, seed germination was delayed and seedlings did not appear until after September 2; therefore the samples of August 26 and September 2 are considered as samples of the fallow plot. The number of *Pratylenchus* in these samples is approximately the same as the number found in the previous soil samples. No decrease during the period of fallow is indicated. Material caught on the 2 screens was kept separate in the processing of these samples and almost all the *Pratylenchus* were found in the coarser material caught on the No. 40 screen. This indicates that the nematodes were in pieces of roots in the soil.

The number of *Pratylenchus* that developed in roots of alfalfa growing in pots of field soil in a greenhouse is in Table 2. These data show that the nematodes in the fallow soil were capable of developing in alfalfa under greenhouse conditions. Roots of these plants weighed from 0.42 to 4.83 gm. per plant. The plants were considerably larger than any of the plants taken from the field and had much more extensive root systems. Discoloration was confined to the smaller lateral roots. The number of nematodes in the roots is comparable to that in the final sampling of spring-sown alfalfa. There is no correlation between the number of nematodes in the soil and in the roots of these plants.

Fall alfalfa.—Samples of seedling alfalfa that was planted August 18 were dug on 4 different dates. The first 22 samples were dug 24 days (September 11) after sowing. Most of the plants had only cotyledons at this time. Their fresh roots weighed from 0.001 to 0.01 gm. per plant. The cotyledons were green and roots were white. *Pratylenchus* were found in only 3 samples. The number of *Pratylenchus* in the fall-sown alfalfa is in Table 1. A second set of 22 samples was dug 13 days (September 24) after the first. The

TABLE 2.—*Pratylenchus per gm. of fresh roots of alfalfa plants in pots of field soil in a greenhouse. Figures are means of 4 pots of 2 plants each*

Date soil was dug	Age of plants in days	*Pratylenchus* per gm. of fresh root		
		North	South	Mean
7/23/53	57	20[a]	254	137
7/28/53	57	54	41	48
8/ 5/53	57	768[b]	1305[c]	1037
8/ 5/53	34	2577[b]	1918[a]	2248
8/11/53	57	1368	68[a]	718
8/17/53	57	1048	24	536
8/26/53	57	1744	838	1291
9/ 2/53	57	552	1308	930

[a] Three pots
[b] Two pots
[c] One pot

roots of these plants weighed 0.01 to 0.06 gm. per plant and were white and healthy appearing. Six yielded *Pratylenchus*. The third set of 22 samples that were dug 19 days (October 13) after the second contained 15 that yielded *Pratylenchus*. These plants had roots that weighed 0.005 to 0.16 gm. per plant. Some light brown discoloration was noticeable. A fourth set of 44 (January 18) samples was dug 107 days after the third. A good stand of alfalfa had been established and the plants were several inches high and had well developed roots weighing from 0.36 to 1.42 gm. per plant. All plants yielded *Pratylenchus*. Although there had been a strong development of lateral roots, many of them were brown. At this time the concentration of nematodes in the roots was approximately the same as at the final sampling of the spring alfalfa.

DISCUSSION.—The large number of *Pratylenchus* found in the roots of spring-sown alfalfa indicates that these nematodes were the primary cause of failure of this planting. None of the other possibly phytopathogenic nematodes found occurred as consistently or as abundantly as the meadow nematodes. Only in occasional samples were there more than 100 per kg. of soil. Further evidence for meadow nematodes being the principal cause of failure is provided by the data from alfalfa planted in the fall. An excellent stand was obtained in the fall, and the number of *Pratylenchus* in the roots of these plants was about 1/1000 of the number in the roots of spring-sown plants of comparable size. The other nematodes were found in similar numbers and as inconsistently in the fall as in the spring.

The difference in the number of nematodes in the roots in the 2 seasons may be the result of a difference in inoculum potential. The data do not indicate the nematode potential in the spring because eggs were not accounted for in the wheat roots. Probably the number of nematodes per unit of soil and roots was higher than indicated. The inoculum potential in the fall is known and probably was lower than in the spring. However, other factors may have affected the susceptibility or tolerance of alfalfa to these nematodes. The alfalfa was growing under quite different conditions in the 2 seasons. The spring plants were growing in cool, wet soil; they were under low light intensity because of shading by the wheat; and they were in competition for nutrients with wheat in soil that had been depleted to some extent by a crop of corn. In contrast to this the fall plants were growing in warm, dry soil; they were under high light intensity; and they had no competition for nutrients in a soil that had received limestone and fertilizer prior to seeding. To what extent the amount of damage to alfalfa caused by these nematodes can be altered by fertility and cultural practices needs further study. The alfalfa in the greenhouse, where the soil was kept moist and temperatures were high, grew more in 2 months, despite rather extensive nematode invasion, than did the plants in the field in the same length of time. The combination of moist soil and high temperature did not occur in either season during the first 2 months after seeding in the field.

Differences in susceptibility to *Pratylenchus* among alfalfa varieties and plants may account for some of the variation in counts. Although there were differences among varieties, there were also wide variations within varieties and all of them were heavily infected. Further study will be needed to determine if there are significant differences in susceptibility among alfalfa varieties and plants.

In addition to seedling damage, the longevity of plants that survive the seedling attack may be seriously reduced by this nematode. Alfalfa is inherently a perennial. However, long-lived stands are relatively rare in Kentucky. The progressive increase in the number of *Pratylenchus* per unit of root and of the percentage of plants infected, shown by the data from the fall plants, indicates that by the time the next growing season arrives there will be a large population of nematodes in the roots to interfere with further development of the plants. Further study is needed to determine to what extent established alfalfa plants can be injured by meadow nematodes.

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THE INHIBITIVE EFFECT OF WATER ON INFECTION BY TOBACCO MOSAIC VIRUS[1]

William B. Allington and Edward F. Laird, Jr.[2]

SUMMARY

Dipping of rubbed leaves in water prior to inoculation was found to inhibit infection of *N. glutinosa* with tobacco mosaic virus. This confirms earlier work by Yarwood. The inhibitory effect is not believed to be the result of either water deficits in the tissue or the "insulating" effect of water films. The inhibition is present in the leaf for at least 24 hours.

In the course of investigations designed to elucidate the nature of infection by plant viruses it became desirable to determine the effect of water on receptivity of wounded tissue. This was a necessary preliminary for proper evaluation of the effects of various solutions on duration of the receptive condition after wounding. Recently Yarwood[3] has reported a deleterious action of water in the virus infection process. Although he offers no explanation of this effect, he infers that it is probably related to water deficit of the plant at time of inoculation. The present paper confirms the results of Yarwood and reports further evidence on the nature of the inhibition involved.

METHODS AND MATERIALS.—This investigation involved the infection of *Nicotiana glutinosa* with tobacco mosaic virus. The number of local lesions produced was considered to be a satisfactory index of the effect of various treatments on the receptivity of the host to the virus.

The leaves were rubbed with a dry abrasive (600 mesh carborundum) without the addition of any virus or water until later. The leaves were then removed from the plants and subjected to water or virus inoculum, or both, by dipping in the appropriate solutions at the desired time intervals after the initial rubbing. The experiments were arranged in a Latin Square design wherever convenient. Tobacco mosaic virus inoculum was prepared by partial purification and concentration by differential centrifugation. All virus was suspended in distilled water.

The general procedure was to remove the leaves from the plants at the time the treatments were made and to incubate the detached leaves with the petioles in pans of water in a constant temperature room held at high humidity and equipped with fluorescent light. In 3 days the local lesion development was usually adequate for obtaining reliable counts.

RESULTS.—*Nicotiana glutinosa* leaves were rubbed after abrasive had been dusted on the surface and after a specified time interval during which they were held in either air or distilled water, they were immersed in the virus inoculum and then rinsed in tap water. Results of 5 typical experiments are given in Table 1. Immersion of the rubbed leaves in water had the immediate effect of reducing the receptivity. After the initial effect became apparent, however, it appears that the rate of receptivity loss was not significantly different between leaves held in water or air before inoculation.

The inhibitive effect of water is apparently very rapid and does not progress beyond a certain point. A series of experiments was undertaken, therefore, to test the possibility of an "isolation effect" of the water.

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[2] Plant Pathologist and formerly Assistant in Plant Pathology respectively.

[3] Yarwood, C. E. 1952. Deleterious action of water in plant virus inoculations. Nature 169: 502.

TABLE 1.—*The inhibitory effect of dipping rubbed N. glutinosa leaves in water before inoculation with tobacco mosaic virus*

Leaf treatments after rubbing and before inoculation	Total number of local lesions on 4 treated leaves[1]										Average lesions per leaf	
	Exp. 1		Exp. 2		Exp. 3		Exp. 4		Exp. 5			
	Air[2]	Water	Air	Water	Air	Water	Air	Water	Air	Water	Air	Water
Dipped in inoculum immediately	201	259	221	251	344	155	208	150	116	189	54.5	50.2
Held in air or water 5 seconds	159	77	104	56	232	60	141	36	82	47	35.9	13.8
Held in air or water 30 seconds	57	45	43	24	44	13	131	34	36	3	15.5	5.9
Held in air or water 5 minutes	12	14	4	7	10	5	31	22	37	3	4.7	2.5

[1] Each experiment consisted of 4 treatments arranged on 4 plants with 4 leaves each in the Latin Square design.
[2] The leaves were held in air or water as indicated here until the indicated time had elapsed before inoculation. These parts of each experiment were made under conditions as uniform as possible with regard to inoculum, etc., in order to facilitate comparisons. The treatments are the same for the figures under "air" and "water" in the first line of this table.

TABLE 2.—*Number of local lesions produced in N. glutinosa leaves by dipping in inoculum for various times after dipping in water*

Treatment after rubbing	Total number of local lesions on 4 inoculated leaves				Average number lesions per leaf
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	
Dipped in inoculum 1 second	135	58	78	82	22
Dipped in water before dipping in inoculum for 1 second	29	26	33	23	6.9
Dipped in water before dipping in inoculum for 30 seconds	34	24	20	15	5.8
Dipped in water before dipping in inoculum for 5 minutes	37	33	29	20	7.4

It was thought that a rapid uptake of water by wounded tissues might coat the receptive cell areas with water films and therby tend to dilute the virus inoculum. It was reasoned that if this actually were the explanation, a prolonged exposure to the inoculum should partially overcome the water effect.

The results presented in Table 2 are from 4 typical experiments designed to test the effect of increasing exposures to the virus inoculum. The techniques used were similar to those used in the experiments reported in Table 1. Here again, exposure of the wounded leaves to water prior to dipping in inoculum drastically reduced the amount of infection. Continued exposures to virus inoculum, however, did not increase nor have a significant effect on the number of lesions produced.

If the inhibitive effect of water were directly related to such factors as turgidity of tissues or water deficit, this effect should be present over a relatively short period of time when the plants are held under normal growing conditions. An experiment was designed, therefore, to test the duration of the inhibitive effect of water. The lower leaves of *N. glutinosa* plants were removed leaving 3 adjacent, fully expanded leaves as near the top of the plant as possible. These leaves were treated in various ways 24 hours before inoculation. Inoculation in this case was accomplished by rubbing the inoculum on the leaves. The results of 5 representative experiments are given in Table 3. In all cases but 1, rubbing the leaves 24 hours prior to rubbing on the inoculum decreased the amount of infection. Without exception the exposure to water immediately after rubbing, but 24 hours before inoculation, reduced the infection still further. During this period the plants were in the greenhouse and under the usual conditions of temperature, moisture, and light which are employed for plant culture.

DISCUSSION.—The inhibition of infection by water of a plant virus is perhaps of considerable significance in studies of the virus infection process. Although the nature of the inhibition is unknown, the results reported here do not appear to support the hypothesis that the effect of water is merely to "insulate" the receptive sites on the cells from the infectious units. Continued exposure of water treated tissue to inoculum did not significantly increase the amount of infection. Because the inoculum is suspended in water, however, it is unwise to make definite conclusions.

The decrease in receptivity of the wounded tissue due to the presence of water is immediate but apparently the normal decrease with time proceeds at about the usual rate with or without added water (Table 1). One confusing aspect of these results is the knowledge that cell fluids are released in the infection area at the time the tissue is wounded. Perhaps these fluids actually have some inhibitory or other effects which have not been detected. Results reported in a previous paper lend support to this hypothesis[4].

The results reported in Table 3 which indicate a considerable reduction in infection due to dipping rubbed leaves in water 24 hours before rubbing with the inoculum, do not indicate that water deficit of the tissues is concerned. It would not be expected that a momentary submersion of a leaf would be reflected in the water content for that period of time. Unless an entirely different effect of water is involved here, one must look further for an explanation of the effects

[4] Allington, W. B., and E. F. Laird, Jr. 1954. The infection of Nicotiana glutinosa with tobacco mosaic virus as affected by potassium nutrition. Phytopathology 44: 297-299.

TABLE 3.—*The average number of tobacco mosaic virus local lesions produced on N. glutinosa leaves when they had been treated prior to inoculation to test the longevity of the inhibitive effect of water on the infection process*

Treatment 24 hours before inoculation	Average No. Local Lesions[1]				
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
Leaves rubbed with no water present	88	12	49	43	53
Leaves rubbed with no water present, then dipped in water immediately	28	6	18	31	23
Leaves untreated before inoculation	104	19	104	53	49

[1] In experiments 1, 2, and 3, 3 plants with 3 leaves each were inoculated for each treatment; each figure, therefore, in these experiments is the average of 9 leaves. Six plants, with 3 leaves per plant, were used in Experiment 4. Ten plants, with 3 leaves per plant, were used in Experiment 5.

observed in the previous experiments. Attempts were made to condition plants to overcome the tendency of wounded tissue to take up water but no success was obtained because free moisture on the leaves complicated the results. Perhaps the water deficit of the leaf cells could be overcome by proper manipulation of the humidity, temperature, light, etc., but the wounded areas still rapidly absorb water, possibly as a result of the capillary attraction from the cell wall surfaces. Water-soaking of these tissues produced erratic results. Sometimes inoculation of water-soaked leaves gave little or no infection but at other times moderate infection resulted. Experiments along this line were not continued because of inability to control the primary factors of interest.

The results presented in this paper are in agreement with those of Yarwood[3] who reported inhibition of tobacco mosaic virus infection on beans by water. The nature of the inhibitory effect of water, however, is unknown.

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THE EFFECT OF A PHYTOPHAGOUS SPECIES OF TETRASTICHUS, NEW TO THE UNITED STATES, ON SWEET CLOVER INFECTED WITH WOUND-TUMOR VIRUS[1]

Shirley S. Teitelbaum and L. M. Black[2]

SUMMARY

A phytophagous chalcid, new to the U. S. A., was identified as *Tetrastichus* sp. near *venustus* Gahan; no other members of the genus in North America are known to parasitize plants directly. It was not observed to parasitize any plant other than sweet clover. Only females were found, indicating the occurrence of parthenogenesis, which is common among other species in the genus.

Sweet clover strains varied in susceptibility to attack. Clones C2 and C6 were susceptible; clone C10 was highly resistant. Many sweet clover plants infected with wound-tumor virus developed stem tumors at the feeding sites of the insect.

An insect, identified as a phytophagous species of *Tetrastichus*, was discovered at the Brooklyn Botanic Garden during an extensive study of diseased sweet clover, *Melilotus alba* Desr. and *M. officinalis* (L.) Lam. The plants were infected with wound-tumor virus, *Aureogenus magnivena* Black (5). Many of these plants growing in the greenhouses exhibited small holes along older sections of their stems and branches and slight swellings in the younger portions near the tips. When the swollen parts of the afflicted stems were cut open, living larvae, pupae, and an occasional adult were seen inside in cavities in the pith. The same type of injury had been observed in 1946 and earlier in sweet clover growing in greenhouses at Princeton, New Jersey, but no associated insect was found. It seems probable, however, that the insect occurs outdoors at Princeton, N. J., and may have been imported to the greenhouse in Brooklyn, N. Y., from there. Before shipping sweet clover clones to the University of Illinois, cuttings were grown in individual cages and only those cuttings of each clone which grew free of *Tetrastichus* were sent there. No *Tetrastichus* has been observed on the many sweet clover plants grown in the greenhouses at Urbana from September 1952 until March 1954.

In order that substantial numbers of adults might be collected, a cheese-cloth cage, 1.5m × 0.7m × 0.3m high, was placed over a group of affected plants and left there for 2 hours during hot sunny days when the temperature in the greenhouse was allowed to reach 100°F. or more. During the first collection, as well as on several subsequent ones, more than 100 adults were found on the cheesecloth inside the cage. Some of the collections were sent to the U. S. D. A. Division of Insect Detection and Identification, where they were identified by Dr. B. D. Burks as a species of *Tetrastickus* near *venustus* Gahan, reported for the first time in the U. S. A. All 292 specimens identified by him proved to be females of the same species, indicating that the insect reproduces parthenogenetically. Parthenogenesis is common among other species of *Tetrastichus*.

The insect may be specific in its host range since it was not found on any other species in the greenhouse. Although closely related plants such as alfalfa (*Medicago sativa* L. var. Grimm) and crimson clover (*Trifolium incarnatum* L.) were grown in large numbers in close proximity to the sweet clover, none ever became infested.

The ability to parasitize sweet clover is in itself a unique characteristic since no other species in North

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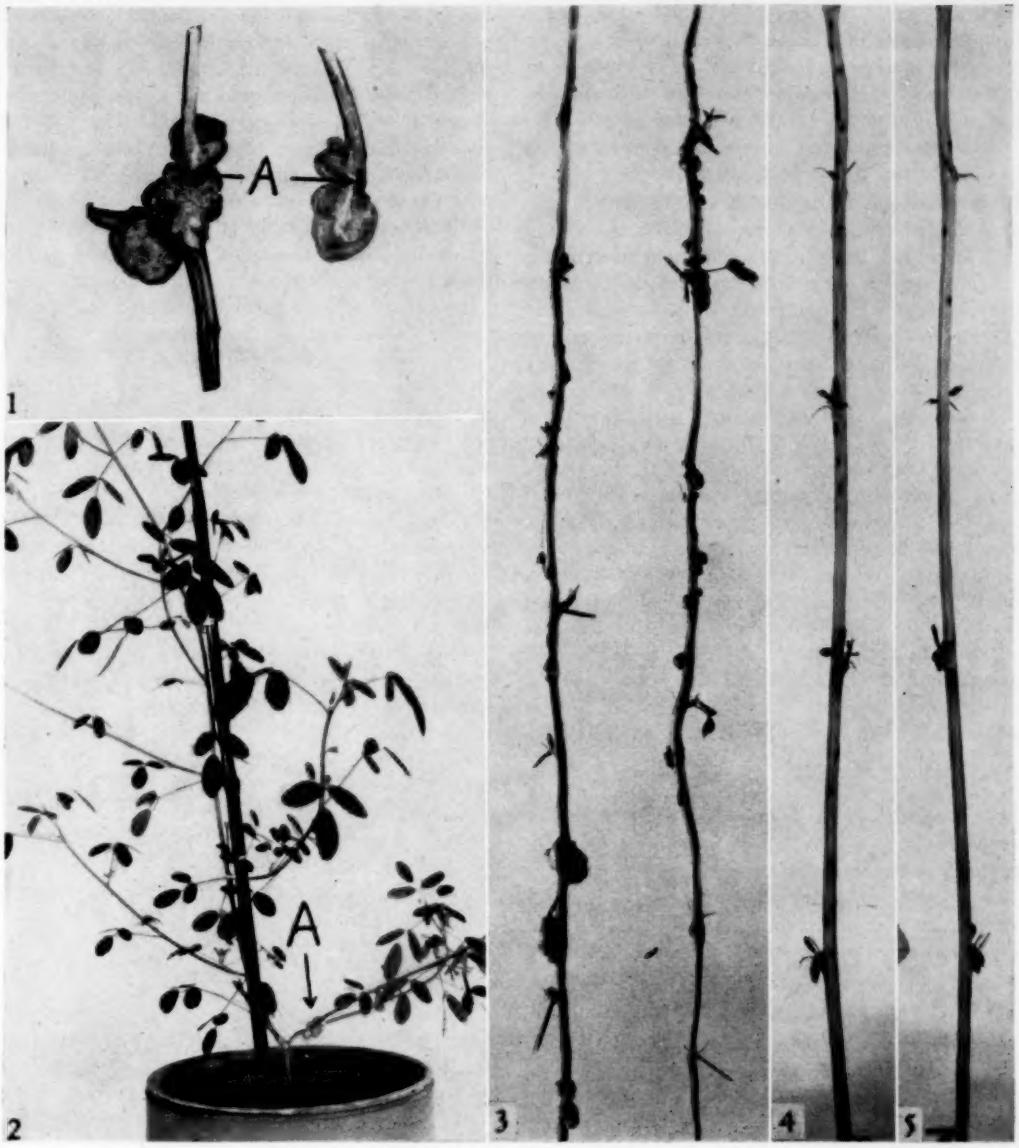

Fig. 1–5. Fig. 1. Tumor from plant photographed in figure 2. The tumor was split open to show hole at "A" where *Tetrastichus* had fed. (×2.4). Fig. 2. Plant grown from a virus-infected cutting of sweet clover clone C6. For a time this plant showed only 1 stem tumor at "A" and in the center of the tumorous growth there was a *Tetrastichus* emergence hole. There were no other emergence holes on the plant. (×0.38). Fig. 3. Selected stems of clone C6 infected with wound-tumor virus and showing an unusually large number of small stem tumors associated with *Tetrastichus* wounds. Some of the small stem tumors appeared to have grown from emergence holes and resembled tumors started from pin-pricks in earlier experiments (1). In the lower part of the photograph an adult *Tetrastichus* may be seen between the 2 stems. (×1.0). Fig. 4, 5. A stem of sweet clover clone C6 noninfected with the wound-tumor virus. No tumors resulted from the insect injury. This stem also showed how the insects emerged from the upper surface of such a horizontal branch. The upper surface of the branch in Fig. 4 had many emergence holes whereas the lower surface of the same branch in Fig. 5 had practically no emergence holes. (×1.0). (Photographs by Louis Buhle.)

America belonging to the genus *Tetrastichus* is known to be phytophagous. Instead of feeding directly upon plants, the other species parasitize insects, which in turn parasitize plants (3). There have been reports of phytophagous species of the genus from other parts of North America, but these have subsequently been shown to be parasitic on insects (4). Although more than 1300 individuals were collected and examined by us in the present study, no other insect species was ever found associated with this *Tetrastichus*. Dr. Burks (4) confirmed our failure to find evidence of any other insects in the stems of infested clover but did find small and large *Tetrastichus* larvae living in the plant tissues. He (4) has also called our attention

to the fact that in 1876 Perris (6) reported a *Tetrastichus* on *Melilotus* in France to which ours may be similar. Perris observed chalcid larvae in cavities in the upper parts of the stems without any indication of gallery or gall formation or the presence of other insects. Only females, about 2 mm. long, emerged in large numbers from his collections of infested stems.

When liberated on living sweet clover, the insects showed a decided tendency to remain near the young growing tips which later developed small swellings. Larval stages, which were relatively immobile, and pupal development were completed entirely within the plant. This is believed to account for the prosperity of this species in the greenhouse in question where very few insect species maintained themselves because of a weekly fumigation with tetraethyl pyrophosphate. Adults emerged from the stems through small circular holes, and lived long enough to reproduce, during which time they apparently did no feeding whatever, and were resistant to dehydration. They usually survived in a test tube without food or water for about 4 days; several individuals lived as long as a week under such conditions. The addition of segments of defoliated sweet clover as a possible source of food did not prolong the life span of the insect. Unfortunately, further information about the details of the life cycle were not obtained due to a sudden, unexplained, and drastic reduction in the number of these insects in the greenhouse.

Sweet clover showed interesting variations in susceptibility to attack by the insect. One clone, C10 (2) was very resistant, whereas 2 others, C2 and C6 were susceptible. A measure of the degree of infestation was obtained by counting the number of emergence holes in the stems. In a study of at least 10 plants of each of these clones grown at the same time under identical greenhouse conditions, it was found that plants belonging to clones C2 and C6 each contained 12 to 13 holes per gm. of stem (9007 holes in 747 gm of C2 stems, 1951 holes in 153 gm. of C6 stems). In C10, a total of only 5 holes was counted in a group of 10 plants, the stems of which weighed 660 gm. The distribution of these 5 holes seemed of possible but unknown significance because they were confined to a single branch of 1 plant.

Examination of affected stems revealed that the holes did not occur around the stem in a random manner. Insects emerged almost exclusively from the upper surface of horizontal branches (Fig. 4 and 5).

The insect had an interesting effect on plants infected with wound-tumor virus. It has been shown that different clones of sweet clover vary in response to infection by wound-tumor virus (2). Some clones, for example, develop a great number of stem tumors whereas other clones only rarely develop stem tumors. C6 is a resistant clone belonging to the latter group. However, many virus-infected C6 plants which were parasitized by the insect developed a much greater number of stem tumors than was ever observed before in that clone and many of these developed in obvious relation to the emergence holes (Fig. 3). For example, 1 resistant C6 plant which was grown from an infected cutting developed a single stem tumor (Fig. 2). Closer examination revealed an emergence hole at the center of the tumor (Fig. 1). There were no other holes and no other tumors present on the plant until considerably later. A correlation between the existence of emergence holes and the development of tumors is not surprising, since other evidence indicates that wounds, whether naturally or artificially incurred, are an important factor in tumor formation in plants affected with wound-tumor disease (1).

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LITERATURE CITED

1. Black, L. M. 1946. Plant tumours induced by the combined action of wounds and virus. Nature **158**: 56.
2. Black, L. M. 1951. Hereditary variation in the reaction of sweet clover to the wound-tumor virus. Amer. Jour. Bot. **38**: 256-267.
3. Burks, B. D. 1943. The North American parasitic wasps of the genus Tetrastichus—A contribution to biological control of insect pests. Proc. U. S. Natl. Mus. **93**: 505-608.
4. Burks, B. D. (personal communications with the authors).
5. Holmes, F. O. 1948. Order Virales. The filterable viruses. Supplement No. 2 Bergey's Manual of Determinative Bacteriology. Ed. 6. The Williams and Wilkins Co., Baltimore, Md. p. 1127-1286 and I-XXIII.
6. Perris, E. 1876. Nouvelles promenades entomologiques. Société Entomologique de France, Annales Series 5, **6**: 223-224.

SYMPTOMS ON TOMATO FRUITS INCITED BY THREE PHYTOPHTHORA SPECIES[1]

P. D. Critopoulos[2]

SUMMARY

Tufts of sporangia on the surface of infected tomato fruits were produced only by *P. capsici*. Immature green fruits were less readily infected by *P. drechsleri* than by the other 2 species. Lace-like and wave patterns were produced by *P. parasitica*.

Inoculations with cultures of *Phytophthora capsici* Leon., *P. drechsleri* Tucker, and *P. parasitica* Dast. were made on detached, unwounded, immature, and mature tomato fruits of 5 varieties in the laboratory to determine whether the symptoms would differ with the species. Tompkins and Tucker[3] found that these species produced similar symptoms when green unwounded fruits were inoculated.

The isolates used were 1) *P. capsici* from foot rot of tomato collected at Stockton, California, 2) *P. drechsleri* from foot rot of kidney bean at Brentwood, California, 3) *P. drechsleri* from buckeye rot of a green tomato fruit at Concord, California, 4) *P. parasitica* taken from buckeye rot of a green tomato fruit at Brentwood, California, and 5) *P. parasitica* from root rot of tomato at Fayetteville, Arkansas.

Fruits inoculated were of the tomato varieties Earliana, Stone, Ponderosa, Sutton's Best, and Pearson. The inoculum was a mixture of zoospores and sporangia in water and was placed directly on the unwounded surface of the fruits. The inoculated fruits were incubated in moist chambers and after different periods of incubation infection was allowed to develop either in the laboratory or outdoors. All infections were confirmed by culture methods.

RESULTS. — Immature green tomato fruits infected with *P. capsici* developed at the point of inoculation firm, diffuse brown spots, which were surrounded by a broad, water-soaked, and light-brown border with ill-defined margin. The decay spread rapidly and the surface was covered with tufts of sporangia. The fruits shrank and became transformed into stone-like mummies in about 12 days. Mature tomato fruits were not readily infected but once the infection started, it proceeded faster than it did on immature fruits. The infected fruits either did not have any browning at the point of inoculation or developed a brownish diffuse discoloration. In the majority of lesions the border was water-soaked, slightly depressed, and sharply demarcated from the healthy tissue. About 8 days after inoculation the surface of lesions on mature fruits was covered with compact tufts of sporangia and occasionally was marked with a yellowish pattern. Mature infected fruits did not become mummified. Of the 3 pathogens used to inoculate tomato fruits, *P. capsici* appeared to be the most vigorous parasite.

Immature fruits were not readily infected by *P. drechsleri*. On those that did become infected, the point of entry of the fungus was marked by a slightly depressed, brown area or brown, irregular, and sharply defined spots, surrounded by light-brown or whitish tissue with well defined border. The brown portion did not enlarge as the infected area enlarged, and the fruit attained a more or less soft consistency. Tufts of sporangia were not produced. Mature fruits were easily infected. At the point where the infection started a brownish discoloration was produced. Most lesions were surrounded by a water-soaked and slightly depressed zone, which was sharply demarcated from the healthy tissue.

Small or full-sized green fruits were readily infected with *P. parasitica*. At the point of inoculation diffuse brown spots or a lace-like brown pattern was produced. The infection spread in a wave pattern. There was a sharp demarcation between diseased and healthy tissue, and as the decay advanced, the fruits became blackish-brown. Mature tomato fruits became readily infected by this species. The lesions had a diffuse brownish center and a broad, water-soaked, slightly depressed advancing margin. Among incipient lesions a few had a smooth surface and their margin was sharply demarcated, whereas others had a corrugated surface and an ill-defined margin. The infection spread faster than in green fruits. On old lesions a wave pattern was well marked. Tufts of sporangia were not produced.

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[2] Asst. Professor of Plant Pathology, University of Salonika, Salonika, Greece.

[3] Tompkins, C. M., and C. M. Tucker. 1941. Buckeye rot of tomato in California. Jour. Agr. Research [U. S.] 62: 467-474.

A PHYTOPATHOLOGICAL NOTE

Observations on the Age of Lodgepole Pine Tissues Susceptible to Infection by Arceuthobium Americanum. FRANK G. HAWKSWORTH.[1] Species of the dwarfmistletoe genus *Arceuthobium* have generally been regarded as incapable of gaining entrance through unwounded host tissues over 3 years old. Weir[2] considers that the resistance of tissues over 3 years old was due to cork formation and the absence of chlorophyll in the bark. By scraping away the dead surface tissues of the bark on twigs which still contained chlorophyll he was able to secure infection on growth as old as 7 years.

Evidence obtained in 1953 on the Roosevelt and Routt National Forests, Colorado, and on the Medicine Bow National Forest, Wyoming, indicates that *Arceuthobium americanum* Nuttall on lodgepole pine (*Pinus contorta* Dougl.) is capable of establishing itself through unwounded bark on tissues over 60 years old. Fig. 1A shows a cross-section through a 10-year-old main-stem infection on 68-year-old lodgepole pine growth and Fig. 1B shows a 23-year-old main-stem infection on 63-year-old growth. Infection occurred on approximately 58- and 40-year-old tissues, respectively. No branches or wounds were visible at the point of infection in either case; subsequent observations on 2- and 3-year-old infections on old tissues confirm that wounding is not a necessary prerequisite to such infection. Dissection of about 50 main-stem infections in one area on the Medicine Bow National Forest indicated that ⅔ of them became established through growth over 5 years old. Infections through old tissues were common on both dominant and suppressed trees.

The character of lodgepole pine bark is believed to be a primary factor enabling *A. americanum* to enter old growth as patches of chlorophyllaceous tissue may be still exposed on 60-year-old stems and, in addition, the bark on some trees of this age was very thin, 1.0 mm. or less in some instances.

This susceptibility or older tissues of lodgepole pine may be an important factor contributing to the extreme destructiveness of *A. americanum* on this tree as a much greater susceptible area per tree is exposed than is believed to be the case for species of *Arceuthobium* on other hosts. Casual observations have shown that other species of *Arceuthobium* are capable of becoming established through unwounded growth over 5 years old but this seems to be rare and of no practical importance in species other than *A. americanum*.

Inoculation experiments with *A. americanum* on various ages of growth of lodgepole pine are being conducted in Colorado to provide more exact information on the infection processes of this mistletoe.—Division of Forest Disease Research, Rocky Mountain Forest & Range Experiment Station, Albuquerque, New Mexico.

[1] Plant Pathologist, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado.

[2] Weir, J. R. 1918. Experimental Investigations on the Genus Razoumofskya. Bot. Gaz. 66: 1-31.

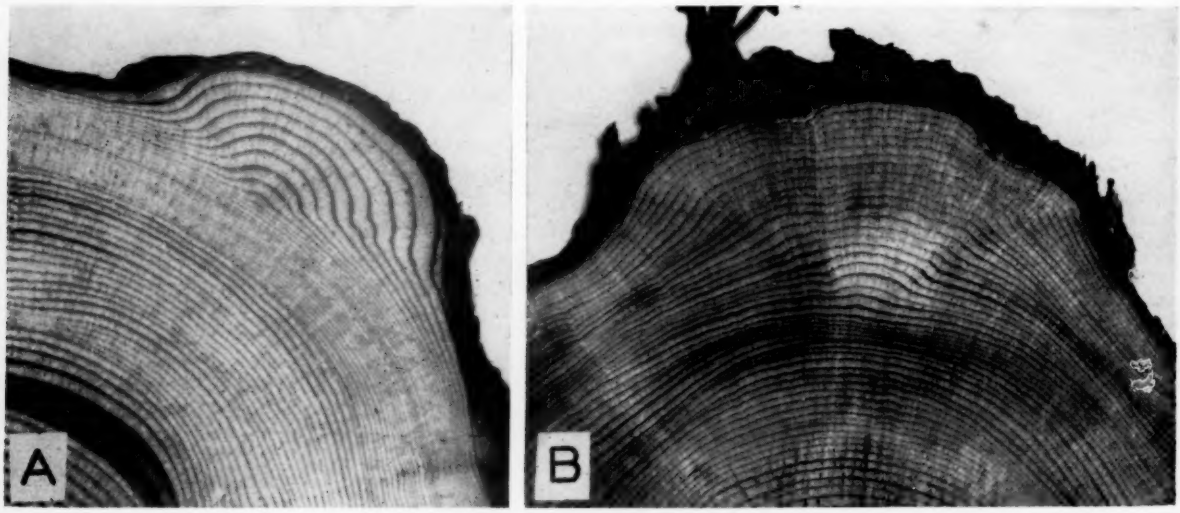

Fig. 1. Cross-sections of dwarfmistletoe infections in lodgepole pine boles. A) infection which became established on 58-year-old growth. (×3). B) infection which became established on 40-year-old growth. (Natural size).